1999 PROGRESS REPORT ON FOOD SAFETY RESEARCH CONDUCTED BY ARS

Compiled and Edited by:

William S. Conway
Beltsville Agricultural Research Center
Building 002, BARC-West
Beltsville, MD 20705

Jane F. Robens
National Program Leader
G. W. Carver Center
Beltsville, MD 20705

James A. Lindsay
National Program Leader
G. W. Carver Center
Beltsville, MD 20705

Agricultural Research Service
U.S. Department of Agriculture
Beltsville, MD
November, 1999

EXECUTIVE SUMMARY

This report summarizes ARS research progress on Food Safety in 1999. The research is categorized into 6 general areas: **I. Control of Food borne Pathogens in Live Animals; II. Pathogen Control During Slaughter and Processing; III. Post-slaughter Pathogen Modeling and Control; IV. Residue Detection and Chemical Analysis; V. Pathogen Control in Manure and Produce; and VI. New Areas of ARS Research.**

I. Control of Food borne Pathogens in Live Animals:
Food safety of animal products begins with management practices, including herd and flock health programs to prevent disease and control infection in the live animal. ARS research to control human pathogens in live animals includes vaccine development, competitive exclusion cultures, and breeding and selection of resistant animals. This research benefits greatly from the long ARS experience with zoonotic pathogens in large animals.

To increase egg safety we need to determine the biological basis for the high susceptibility of adult hens to SE during feed withdrawal which is used to induce molting and increase subsequent rates of laying. Using *in vitro* tests for functional responsiveness and efficiency of heterophils ARS demonstrated that feed withdrawal alters the number and function of peripheral blood heterophils. This knowledge points the way to using immune lymphokines to reduce the susceptibility of molting hens to SE and thus increase egg safety.

Although the incidence of *Salmonella* and *Campylobacter* in broilers has been decreasing, further measures are needed to assure an even safer food supply. ARS conducted a nationwide epidemiology study of *Salmonella* and *Campylobacter* in broilers to: identify the most importance sources of *Campylobacter* and *Salmonella* in the production environment. e.g., paper pads; water; litter; feed hoppers; transport crates, mice; wild bird and animal feces, and insects; and determine intervention strategies that effectively reduce transmission and limit consumer exposure to *Campylobacter* and *Salmonella.* The results of this study has allowed development of a commercial scale trial that targets 4 control points to further reduce the levels of pathogens on poultry; these are hatching cabinet disinfection, consistent use of new paper pads for newly hatched chickens, competition exclusion, and litter treatment in the production house.

Epidemiological studies from production to consumption are needed to identify intervention measures for control of *Salmonella* in swine. ARS developed computer simulation models to determine the effect of intermittent pathogen shedding on accurate estimation of population prevalence. Results suggested that simple random sampling protocols will underestimate the true prevalence. This information will help to develop protocols that can more accurately identify potential critical control points for effective control of *Salmonella* from farm to slaughter plant.

Rapid methods are needed to identify multi-drug antibiotic resistant bacteria, such as, *Salmonella typhimurium* DT104 in food producing animals and in their environment. ARS determined that the resistance to florfenical and chloramphincol are conferred by the *flo* gene. Presumptive identification of this multiresistance can now be made rapidly based on the presence of the gene and its resulting phenotype. This information will speed the development of a rapid test kit which will enable identification of DT104 in a timely manner and slow its spread to food products.

Slaughter and processing equipment for the broilers must be optimized to prevent contamination from gut pathogens reaching surfaces of their edible products during the processing. ARS developed an easily visually identifiable food grade marker that can be used in commercial facilities to investigate the possible leakage of gut contents from the upper gastrointestinal tract. Results from 5 commercial broiler processing plants showed that over 60% of the carcasses were contaminated with the marker following feather removal. Use of this technique can lead to the design and selection of new processing equipment that will minimize leakage of gut contents and increase the safety of broiler meats.

Competitive exclusion cultures were developed that control *Salmonella* and other enteropathogens in swine. This culture significantly reduced mortality in baby and weaned pigs, and in addition significantly reduced mortality associated with pathogenic *E. coli* in very early weaned pigs. The individual bacteria have been isolated and a recombined culture has been developed that has the same efficacy as the original culture. Specific identification of the bacterial components of such cultures is necessary to allow reproducibility of results and for FDA approvals.

The efficient way to control epizootic infections in food producing animals and poultry and prevent transmission of pathogens to food products is to develop genetic resistance. Using challenge tests to many lines of pigs, ARS identified several inbred pigs which were resistant to the foodborne parasite *Toxoplasma gondii.* More detailed genotyping studies can now be conducted with these animals to determine the genes which endow them with parasite resistance. The genes can then be used as selection markers for breeding.

ARS research to characterize the extent and distribution of *Cryptosporidium parvum* oocysts in Chesapeake Bay indicates that oysters from all bars being monitored are contaminated and that the oocysts are viable. Sequencing of the PCR products from some oysters indicates that the oocysts are *C. parvum*, bovine genotype. A real-time fluorescent-probe based PCR assay method developed for the detection of *C. parvum* oocysts provides results in under 2 hours, with a detection limit of 5 to 100 oocysts depending on whether the water is clear or turbid. By detecting *C. parvum* in oysters ARS has shown that shell fish can be good indicators of fecal pollution of surface waters and that they pose a public health risk if eaten raw. Utilizing PCR testing for detection of *Cryptosporidium* increases our ability to detect lighter infections and to differentiate species infectious for humans versus those noninfectious for humans and in some cases to determine the source of the fecal contamination.

II. Pathogen control during slaughter and processing:
Slaughter and processing are key links in the food safety chain. Improved understanding of food borne pathogen transmission, and control steps in prevention, sanitation, and processing technology are necessary elements to prevent contamination, cross contamination and wide spread food borne illness.

ARS scientists modified a commercially available LAL test (a colorimetric test which detects the presence of lipopolysaccharide, LPS from the Gram negative bacterial cell wall) to allow determination of the level of microbial contamination on a hot or chilled beef carcass within 15 minutes. The test was sensitive to aerobic bacterial levels as low as 100 cfu/cm^2 and correlated well with standard plate counts of surface swab samples, whether the carcass surface menstruum was predominantly lean fascia or adipose fascia. This assay provides manufacturers with a tool to rapidly gauge carcass contamination, and rectify potential problems in the processing line.

Studies compared conventional solid to elevated wire mesh flooring during transport and holding of poultry broilers prior to slaughter. Results indicated that although broilers transported and held on solid flooring had noticeably dirtier breast feathers and higher coliform and *E. coli* counts prior to scalding and defeathering, bacteria recovery from external carcass rinses did not differ between the solid and wire flooring treatments after defeathering. The processing steps of immersion scalding and defeathering consistently lowered carcass bacteria counts and the incidence of *Campylobacter*-positive, but not *Salmonella*-positive carcasses.

Broiler processing was examined to determine the microbial profile of carcasses, specifically in relation to *Campylobacter* levels. While scalding dramatically lowers the population of *Campylobacter*, significantly more *Campylobacter* is recovered from carcasses sampled after defeathering than those sampled before (directly after scalding). As carcasses continue to move through the process after de-feathering, the recovery of *Campylobacter* is lessened. An area of the plant, specifically the picker, has been identified as leading to heightened recovery of *Campylobacter*. This information may now be used by industry and the FSIS to help identify a critical control points, or suggest where application of sanitizer technology may be used.

Green, yellow and cyan fluorescent strains of *C. jejuni* have been constructed and used to identify locations of attachment of *Campylobacter* to chicken skin. Studies indicate that the bacterium binds to specific phospholipids and proteoglycans present in the skin epidermal and dermal layers. This research technology will aid in understanding the basis of microbial attachment and detachment to animal carcasses, and assist in evaluating technologies for carcass decontamination.

Campylobacteriosis is recognized as a major human foodborne disease agent in the USA. ARS studies indicate that pork can be a vehicle for *Campylobacter* that could ultimately rival poultry as a significant exposure source for humans. The studies provide base-line data for the incidence of the bacterium on swine carcasses and during carcass dressing, and will assist the FSIS if performance standards for *Campylobacter* spp. on red meat are introduced.

III. Post Slaughter Pathogen Modeling and Control:

Risk assessment is fundamental to evaluating the effect of production practices, processing and transportation systems on the contamination of food producing animals and plants. Rapid and accurate methods of detection, and quantitative measurement of pathogens at all critical points during food processing are needed to provide the necessary data to carry out risk assessment, to develop and validate predictive microbiological models, and to identify areas where interventions are most critically needed.

A farm-to-table simulation model for assessing the risk and severity of *Salmonella* spp. and *Campylobacter jejuni* infections from chicken was developed, and a case study was created to demonstrate how the model can be used to make food safety decisions. The model was incorporated into Poultry-FARM for use by industry and FSIS at the processing plant to assess the public health impact of individual lots of chicken destined for specific distribution channels and consumer populations.

Research showed that a sensitizing cold treatment prior to thermal processing might increase the effectiveness of the thermal process for the destruction of pathogenic bacteria. Prior cold shock resulted in a reduction in the thermal tolerance of *L. monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella typhimurium* in juices. The inducible and enhanced vulnerability to heat in model and food systems shows the potential for this approach to be used by the food industry as a practical and efficacious post-processing intervention strategy to eliminate these bacterial pathogens. Cold shock prior to pasteurization of fruit juices could provide an extra measure of safety and would also allow juice processors to reduce thermal processing requirements for pathogen control, while maintaining the fresh qualities of the juice.

Diarrheal disease caused by *Salmonella typhimurium* DT104 is associated with greater morbidity and mortality than infections caused by other *S. typhimurium* strains. A comparison of the ability to invade mammalian cells, and of the stress tolerance of *S. typhimurium* DT104 to other *S. typhimurium* strains in a variety of foods was examined. Studies indicated that measures taken to inactivate or inhibit the growth of *Salmonella* in food should also be sufficient to inhibit *S. typhimurium* DT104.

Studies were performed to determine the heat treatment required to kill *Listeria monocytogenes*. The data was used to develop a mathematical model for determining the effects of environmental parameters on thermal resistance of *L. monocytogenes*. Using this predictive model, food processors should be able to design thermal processes for the production of a safe food with extended shelf life without substantially adversely affecting the quality of the product.

Immunomagnetic separation methods were developed for the rapid detection of *Campylobacter* spp. in poultry products. Polyclonal anti-*Campylobacter* antibodies conjugated with biotin were used to coat streptavidin-labeled magnetic beads, while the same antibodies were also used to coat tosylactivated beads. The beads, with their bound *Campylobacter* can be magnetically isolated

from other sample material and microorganisms, and represents a new and innovative approach for extracting, concentrating and isolating *Campylobacter* spp. directly from foods without enrichment

A procedure to rapidly detect the *E. coli* in beef hamburger patties was developed. Streptavidin coated magnetic beads and biotin-labeled anti *E. coli* O157 antibodies were used to capture the bacteria which were further immobilized on biotinylated nitrocellulose membrane sticks. The bacteria were further treated with fluorescein-labeled anti *E. coli* O157 antibodies and urease conjugated anti fluorescein antibody. Urease catalyzed production rate of NH_3 from urea was then determined. The approach allows detection of *E. coli* O157:H7 at a level of ~1 CFU/g after a six-hour enrichment. The approach may be applied for field use, that is, sample collection from remote locations away from central laboratories, since the immobilized bacterial samples are stable for 48 h at 4C without significant changes in signal.

Hydrodynamic pressure (HP) process uses a high-energy explosive to generate a supersonic shock wave. The process was investigated for use as a method for reducing the microbial load in fresh and prepacked meats. HP treatment resulted in a significant reduction in APC indicating that the process was effective in reducing normal spoilage microorganisms found in fresh and temperature-abused meats, which could extend the shelf life of meats and meat products.

ARS research resulted in the FSIS issuing a message to consumers that " consumers should not eat ground beef patties that are pink or red in the middle unless a food thermometer has been used to verify cooked temperature". Subsequent surveys indicate that most consumers either do not own a meat thermometer or if they own one, do not use it in cooking meat products. Thus, additional joint ARS-FSIS studies on premature brown color in cooked beef patties were conducted, focussing on: (1) the use of outdoor gas grills as the cooking equipment and, (2) temperature and color changes post-cooking and before consumption. Results indicated that internal color of patties prepared and cooked under these conditions is even less reliable as an indicator of food safety. A workshop held in Washington, DC in November, 1998 resulted in the formation of a Food Temperature Indicator Association to conduct research and perform consumer affairs regarding temperature devices. Also several major retailers, for example, Giant have initiated ground beef thermometer usage programs employing ARS-FSIS research findings on safe-cooking of beef patties.

IV. Residue Detection and Chemical Analysis:
Food safety research also includes the detection of residues of drugs, environmental toxins, natural toxins, and chemical analysis of nutrient quality.

A collaborative study, Determination of Pesticide Residues in Nonfatty Foods by SFE and GC/MS, was completed through the AOAC® Official Methods Program. Collaborators included 17 laboratories from 7 countries, and evaluated commodities such as apples, green beans, and carrots which contained a total of 23 pesticides. The methodology used is expected to meet the criteria for global acceptance of most of the pesticides examined.

GC-Pulsed Flame Photometric Detection (PFPD) was evaluated for the detection of OPPs in pork fat and ground meat. The extraction procedure was the same as the FSIS CHC3 method used for the analysis of OCPs in fat. Results verified that the OPPs (and other classes of pesticides) are also present in the same collection fraction as the OCPs obtained by the FSIS CHC3 procedure. The analysis of OPPs in the extracts using GC/PFPD was found to be reproducible and provide exceptionally low limits of detection. Currently, FSIS only uses GC-electron capture detection (ECD) in this analysis for the halogenated pesticides that does not detect nonhalogenated pesticides, such as many OPPs. The EPA has requested that FSIS also analyze OPPs to help meet goals outlined in the Food Quality Protection Act. The results of this project indicate that FSIS may use the PFPD with their existing method to expand the range of analytes tested in their monitoring program employing the same sample extract, without the need for additional extraction techniques. These studies impact the capabilities of regulatory and other laboratories that analyze pesticide residues in food. The use of lower cost, more rapid, easier, and higher quality methods can increase the monitoring rate, better ensure the absence of residues, and provide more accurate data for risk assessment purposes.

ARS has a focussed program for the develop inexpensive, rapid methods for the detection of dioxins and related compounds in foods. An immunoaffinity column (IAC) generated from a monoclonal antibody has shown promise as a convenient cleanup for polychlorinated dibenzo-*p*-dioxins (PCDDs) from serum samples. Studies showed that serum could be directly applied to the column, several of the most toxic PCDDs bound to the IAC, and the congeners could be readily eluted before analysis by high resolution gas chromatography-high resolution mass spectrometry. Addition of a prewashing step before applying the sample, dramatically increased the recovery of 2,3,7,8-TCDD increased from 16% to 83%. Verification studies of samples provided by the CDC indicated that six dioxin and furan congeners were quantitated within the 95% confidence limits of the method at the part-per-quadrillion (ppq) level. This simpler technology will be of importance to regulatory agencies and other scientists for reducing the time and costs associated with dioxin analysis, presently $1000/sample.

Polyclonal antibodies against ractopamine-hemigluterate-KLH were generated and showed sensitivity to ractopamine at the ppb level. Ractopamine HCl is a β-adrenergic agonist which is an active "leanness-enhancing" agent in several economically important livestock species. The antibodies could be used by a variety of regulatory organizations to monitor for illegal use of this β-agonist.

V. Pathogen Control in Manure and Produce

The efficacy of commercial brush washing equipment, used in conjunction with conventional and experimental washing and sanitizing agents, in decontaminating apples was investigated in a commercial cider mill operated by the FDA in Placerville, California. Results indicated that brush washing in the presence or absence of washing and sanitizing agents did *not* significantly reduce the

bacterial population on apples or in cider. Cider contamination probably resulted from the inability of such washing systems to inactivate or remove microorganisms in inaccessible calyx and stem areas of apples.

An agreement between ARS and Pennsylvania State University (PSU) was negotiated to facilitate the design, fabrication and evaluation of a BL-2 fruit and vegetable processing research facility. Plans for short-term research to develop new decontamination technology and solve design problems limiting further development of the system were initiated. Collaborative research in this area with Penn State and acquisition of a BSL-2 facility will give ARS a unique capability for improving the microbiological safety of fresh produce

Studies were conducted to determine whether areas of decay in grounder apples ("drops") may be a potential source of *E. coli* O157:H7 in apple cider made from such fruit. Wounds in fresh apples inoculated with this human pathogen and one of two decay fungi, *Penicillium expansum* or *Glomerella cingulata* indicated that the *P. exapansum* fungus was antagonistic to the bacteria. However, apple wounds containing *G. cingulata* and *E. coli* O157:H7, showed extensive bacterial growth. Since the extent of growth of *E. coli* O157:H7 in the presence of *Glomerella* would be sufficient to contaminate a large quantity of cider, these results clearly demonstrate the potential risk of using decayed apples for production of unpasteurized cider.

Research on novel combination treatments for decontamination of alfalfa seeds was conducted, and several alternative treatments were realized. A combination of treatments using gamma irradiation followed by chemical sanitizing with 2% calcium hypochlorite yielded a 5 log reduction in the population of *Salmonella* on inoculated seeds, a reduction level that could not be achieved with the individual treatments alone. The efficacy of 20,000 ppm chlorine in sanitizing seed contaminated with *E. coli* O157:H7 was confirmed. Data generated on the use of 2% calcium hypochlorite was used in support of a successful joint USDA/industry petition to the EPA to allow use of this agent for sanitizing seed destined for sprouting. This technology is now widely used in the U.S. sprouting industry. The combination treatment strategy should enable sprout growers to achieve FDA goals with current technology if there are no unforeseen regulatory restrictions or economic constraints.

VI. New Areas of ARS Research:

ARS scientists conduct research that is both basic and applied. Innovation is a key attribute, and new areas of study are constantly being undertaken. Several new areas of research were initiated:

Determining molecular characters for strain-genotype identification and diagnosis of *Toxoplasma, Cryptosporidium, Cyclospora* and other parasites of importance as food or waterborne pathogens, which otherwise threaten food products or reduce production efficiency.

Evaluating the feasibility of hyperspectral imaging and other technologies for identification of fecal and crop ingesta surface contamination on poultry carcasses and to develop a real time on-line imaging system for contaminate identification.

Food safety engineering research consortium with Purdue University focusing on development of: diagnostic tools for rapid identification of biological and chemical foodborne contaminants; models to predict and track foodborne contaminants; identification, design, and evaluation of alternative processing, handling, packaging, transport, and storage systems to minimize and/or reduce food contaminants; and technology transfer of information and knowledge related to food safety for the food industry, government agencies, academia, and the public.

Conclusion:

ARS has an organized and productive research program on food safety of both animal and plant products. This research addresses prevention and control of pathogens, and residues at every aspect of the **farm to table continuum**; from prevention in the live animal or growing plant, to techniques of eliminating pathogens at the processing level, to storage, quality and the preparation of food products. The ARS research program in food safety is of vital importance to FSIS, and supports their efforts to continue to provide the consumer with the safest of food supplies.

1999 PROGRESS REPORT ON FOOD SAFETY RESEARCH CONDUCTED BY ARS

TABLE OF CONTENTS

Table of Contents

PART I. CONTROL OF FOODBORNE PATHOGENS IN LIVE ANIMALS

CYTOKINE-MEDIATED MODULATION OF THE INNATE IMMUNE RESPONSE TO PREVENT SALMONELLOSIS IN POULTRY

ARS Contact Persons:	CRIS NUMBER:	6202-42000-008
M.H. Kogut, L.H. Stanker	FSIS CODE:	C1MA02
R.L. Ziprin	CRIS TERM. DATE:	April 2000

Food and Feed Safety Research Unit
Food Animal Protection Research Laboratory
College Station, TX
Phone: 409-260-9484
Fax: 409-260-9332
E-mail: kogut@ffsru.tamu.edu

OBJECTIVE A: Develop a non-traditional immunological method to prevent and/or control salmonellosis in poultry.

PROGRESS A: During the past year, studies were undertaken to determine the effects of induced molt on basal functional activities of heterophils from aging hens. For this purpose, heterophils from both molted and unmolted hens were examined by *in vitro* bioassays for functional responsiveness and efficiency. The results indicated that feed withdrawal to induce molt alters the number and function of peripheral blood heterophils. This decreased efficiency of heterophil functional activity appears to play a role in the increased susceptibility of molting hens to *Salmonella enteritidis* infections. Preliminary investigations conducted during the last year demonstrated that the oral administration of *Salmonella enteritidis*-immune lymphokines (SILK) to hens 24 h before feed withdrawal for molting will significantly reduce the susceptibility of the hens to SE infections. Mechanistically, the increased resistance induced by the SILK appeared to be mediated through the activation of circulating heterophils. These results suggest that SILK could become a commercial product for hens during induced molt that can convey protection against *Salmonella enteritidis* infections.

IMPACT/TECH TRANSFER A: The successful demonstration that SILK will also induce protection in adult hens during a period of high susceptibility to SE provides further proof that this technology can be used in all phases of the poultry industry without apparent harm to the birds. The technology is extremely important because it provides a safe, natural alternative to control of *Salmonella*; thus reducing or eliminating the need for antibiotics.

OBJECTIVE B: Optimize an effective delivery system(s) of SILK to neonatal poultry.

PROGRESS B: This year tests were performed to evaluate whether SILK could be administered to day-old turkeys by routes routinely used by the industry for vaccines and still be able to confer protection to the birds against localized enteric *Salmonella* infections. The results indicated that the delivery of SILK either orally, subcutaneously, or by aerosol confers significant protection against salmonellae infections that persists for up to six days. The mechanisms appear to be via SILK-mediated activation of heterophil function.

IMPACT/TECH TRANSFER B: These experiments suggest that SILK could be readily administered to poultry without new specialized equipment. We are working with Cobb-Vantress and Ross Poultry Breeders to analyze the most cost effective route to administer SILK for use by the poultry industry. Additionally, we began discussions with British United Turkeys, Inc. to initiate studies of use of SILK in turkeys.

OBJECTIVE C: Identify and purify the effector cytokine(s) and clone the gene for mass production. Identify and clone genes for avian cytokines for use in the immunopotentiation of host immune responses in neonatal poultry.

PROGRESS C: We conducted studies to identify the specific protein in SILK responsible for the protective effect in chickens. SILK was fractionated by ammonium sulfate precipitation and the protective activity was recovered in the 40-60% ammonium sulfate saturation fraction. Monoclonal antibodies against SILK were then linked to a solid matrix to form an immunoaffinity column over which the 40-60% fraction was run. Four peaks of bound protein were eluted. The portion containing the protective activity was recovered and was then subjected to size exclusion, ion exchange, and hydrophobic interaction chromatography. In addition to these studies on fractionation of SILK, purified recombinant chicken interferon-gamma (IFN-g) was produced after the genes were cloned into *E. coli.* We demonstrated that the recombinant chicken interferon-gamma (rChIFN-g) was capable of enhancing the functional activities of heterophils from day-of-hatch chicks. These results illustrate that the heterophils from neonatal chicks possess the receptor for IFN-g; thus, this cytokine could possibly be used either as an immunopotentiator for day-old chicks or as an adjuvant for vaccines. Preliminary experiments showed that rChIFN-g induced significant protection against SE organ invasion in day-old chickens and turkeys. These result suggest that one major component of SILK is IFN-g.

IMPACT/TECH TRANSFER C: Isolation of the SILK protein will enable us to clone the gene for this protein. The cloned gene will be a useful tool for the development of economical and effective immunologically-based treatments for the reduction of *Salmonella* in poultry products. Purification of the SILK and/or isolation of the gene(s) of SILK and other avian cytokines will lead to the licensing of this technology.

OBJECTIVE D: Determine the effect of SILK on the incidence of horizontal transmission of *Salmonella* in neonatal chickens and turkeys.

PROGRESS D: The effect of SILK on horizontal transmission of *S. arizonae* (SA) in turkey poults and *S. gallinarum* (SG) in broiler chicks was assessed in a seeder/contact model. Seeders were challenged with the appropriate bacterium and contacts were either untreated or administered SILK. Seeders and contacts cohabited within an experimental group throughout the experiment. There were no differences in mortality between nontreated and SILK-treated contact turkeys. However, nontreated contact chicks had a mortality rate of approximately 68% whereas significant reduction in mortality was observed in the SILK-treated contact chicks (15%). Horizontal transmission, determined by organ invasion, of SA to contact turkey poults and SG to contact broiler chicks was also significantly reduced by SILK.

IMPACT/TECH TRANSFER D: SILK significantly reduces the horizontal transmission of *Salmonella* in poultry. Horizontal transmission of *Salmonella* is the most common method of spread of these bacteria in neonatal poultry. Commercial breeder flocks and broiler hatcheries have been identified as sources of salmonellae. Unfortunately, hatchlings are most susceptible to salmonella colonization and organ invasion. The colonization dosage of salmonellae for day-of-hatch chicks is 100 times less than that of 5 day-old chicks. The results from these experiments suggest that *in ovo* treatment of poultry with SILK can induce a significant protection against *Salmonella* immediately upon hatch.

OBJECTIVE E: Evaluate efficacy of SILK on *Salmonella typhimurium* DT 104 in neonatal chickens.

PROGRESS E: DT 104 has emerged as an increasing cause of *Salmonella* infections in the United Kingdom with increasing number of cases being diagnosed in the US. DT 104 isolates are highly resistant to antimicrobial agents demonstrating a pattern of resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. These experiments were undertaken to determine whether SILK could increase the resistance of chickens to colonization with this zoonotic bacterium. The results demonstrated that the administration of SILK significantly reduced both organ invasion and cecal colonization of DT 104 in neonatal chickens.

IMPACT/TECH TRANSFER E: The successful induction of resistance to DT 104 to neonatal chickens suggests that an immunologically-based preventive strategy against all *Salmonella* in poultry could be a significant intervention strategy for the poultry industry.

PUBLICATIONS:

Kogut, M.H., C. Holtzapple, V.K. Lowry, K. Genovese and L.H. Stanker. 1998. Comparison of the functional activation of neonatal chicken and turkey heterophils in response to stimulation by inflammatory agonists. Am. J. Vet. Res. 59:1404-1408.

Genovese, K.J., V.K. Lowry, L.H. Stanker and M.H. Kogut. 1998. Administration of *Salmonella enteritidis*-immune lymphokine to day-old turkeys by subcutaneous, oral, and nasal routes: a comparison of effects on *Salmonella enteritidis* liver invasion, peripheral blood heterophilia, and heterophile activation. Avian Pathol. 27:597-604.

Lowry, V.K., G.I. Tellez, D.J. Nisbet, G. Garcia, O. Urquiza, L.H. Stanker and M.H. Kogut. 1999. Efficacy of *Salmonella enteritidis*-imune lymphokines on horizontal transmission of *S. arizonae* in turkeys and *S. gallinarum* in chickens. Int. J. Food Microbiol. 48:139-148.

Genovese, L.L., V.K. Lowry, K.J. Genovese, J.R. DeLoach and M.H. Kogut. 1999. Enhancement of phagocytosis and bacterial killing by heterophils from neonatal chicks after administration of *Salmonella enteritidis*-immune lymphokines. Vet. Microbiol. 65:133-143.

PATENTS:

Method to Produce Granulocyte Colony Stimulating Factor From Immortalized Avian T Lymphocytes and Method to Produce Immortalized Cells. Patent Number 5,841,443.

EPIDEMIOLOGY AND ECOLOGY OF SALMONELLA ENTERITIDIS IN COMMERCIAL POULTRY FLOCKS

ARS Contact Persons:
D.E. Swayne, R.K. Gast,
J. Guard-Petter,
P.S. Holt, B. Mitchell

CRIS NUMBER: 6612-42000-022
FSIS CODE: S1AM01
CRIS TERM. DATE: April 2001

Southeast Poultry Research Laboratory
934 College Station Road
Athens, GA
Phone: 706-546-3433
Fax: 706-546-3161
E-mail: dswayne@seprl.usda.gov

OBJECTIVE A: Determine whether intestinal colonization and organ invasion by *Salmonella enteritidis* (SE) can persist into adulthood following the oral inoculation of newly hatched chicks.

PROGRESS A: The effectiveness of efforts to reduce egg-transmitted SE infections in humans is largely dependent on controlling the incidence of this pathogen in commercial laying flocks. Identifying the sources of introduction of SE into laying flocks is essential for developing effective control strategies. To investigate the potential for SE infections acquired by newly hatched chicks to persist until the age at which hens began to lay eggs, groups of chicks were given an oral dose of a phage type 13 SE isolate at one day of age. Although SE was found in the livers, spleens, and ceca of all sampled chicks at one week after inoculation, colonization generally persisted beyond four weeks post-inoculation only in the ceca. Nearly half of the remaining hens were still shedding SE in their feces at 24 weeks of age, and one of the 62 hens laid two eggs that were internally contaminated with SE during the initial 4-6 weeks of egg production.

IMPACT/TECH TRANSFER A: Chickens exposed to SE shortly after hatching can apparently remain infected until maturity, at which time they might produce contaminated eggs or spread the infection to other susceptible, previously unexposed hens. The results of this study suggest that efforts to detect and control SE infections in young poultry can indeed have direct relevance to the overall goal of reducing egg contamination.

OBJECTIVE B: Determine whether negative air ionization can reduce airborne horizontal transmission of *Salmonella enteritidis* (SE) between groups of chicks in isolation cabinets.

PROGRESS B: Airborne movement of SE can lead to the transmission of infection between groups of chickens that are not in direct contact with each other. Dust is a leading candidate for mediating this type of transmission. Electrostatic space chargers can reduce levels of circulating airborne dust particles by imparting a negative charge that causes them to be attracted to grounded surfaces. To

determine whether this could affect the airborne transmission of SE, chicks were housed in controlled-environment isolation cabinets in which airflow was directed across an unoccupied central area from an "upstream" group of birds to a "downstream" group. Negative air ionizers were installed in some cabinets. Groups of chicks were placed in the upstream ends of the cabinets and inoculated orally with SE at one week of age. On the following day, one-day-old chicks were placed in the downstream ends of the cabinets. When chicks were sampled at three and eight days post-inoculation, SE was found on the surface of 90% of the downstream chicks from cabinets without negative air ionizers, but on only 40% of the downstream chicks in the presence of the ionizers. Similarly, SE was recovered from the ceca of 53% of sampled downstream chicks in cabinets without ionizers, but from only 1% of the ceca of chicks in cabinets where ionizers were installed.

IMPACT/TECH TRANSFER B: Electrostatic space chargers, by restricting the circulation of contaminated dust particles, can reduce the airborne movement of SE and the horizontal spread of SE infection between chickens in isolation cabinets. The application of negative air ionization technology could thus contribute to controlling the incidence of SE infection in commercial poultry flocks.

OBJECTIVE C: Determine effectiveness of an electrostatic space charge system for reducing airborne *Salmonella* and dust in a caged layer room and in commercial hatching cabinets.

PROGRESS C: Earlier experiments have shown that SE can be easily transmitted through the air from a group of infected birds to a group of uninfected birds. The electrostatic space charge system used in a caged layer room reduced artificially generated dust concentration from 72 to 91% and naturally generated dust concentration from 50 to 54%. The system reduced airborne SE by approximately 95% over a 10-day sampling period. A custom-built electrostatic space charging and collection system reduced dust in a commercial hatching cabinet by an average of 83%. *Salmonella* positive cabinets were reduced up to 83%. Hatchability with the system improved an average of 2.7% compared to an untreated control cabinet.

IMPACT/TECH TRANSFER C: That the system was able to reduce airborne levels of SE by 95% while reducing only about 52% of the dust is not surprising since earlier studies have shown that treatments which reduce airborne dust by a factor of 2 reduced airborne bacteria by a factor of 100 to 3000. Results of these studies have generated interest from several large poultry integrators for pathogen reduction in their hatcheries. A patent application is pending for the technology and three license applications have been received for manufacture of the system.

OBJECTIVE D: Produce a mutated strain of *Salmonella enterica* serovar Enteritidis that will delineate the maximum amount of low-molecular-weight lipopolysaccharide that can be produced.

PROGRESS D: After producing a *wzz* mutant of serovar Typhimurium by transposon mutagenesis, procedures were developed for transferring this mutation and others into the chromosome of serovar Enteritidis. The gene *wzz* encodes an enzyme that determines chain length. Mutations in this gene

abolish the ability of Enteritidis to produce high-molecular-weight O-chain LPS, which is a structure produced in quantity by only a few natural isolates. The *wzz* mutant was inserted into a strain that could grow to high cell density, which is a capability required for serovar Enteritidis to efficiently contaminate eggs. Some animal studies have been completed, and so far they indicate that mutation of *wzz* does not affect baseline invasion. Instead, this mutation lowers variance in the population of bacterial cells. The biological consequence of decreased variance due to a loss of *wzz* can only measured by administering the pathogen parenterally rather than orally. Compared to wildtype, mutation of wzz resulted in a milder infection, a lower recovery of organisms from internal organs and diminished mortality in 20-day old chicks infected with 5×10^6 CFU. This experiment confirms that high-molecular-weight O-chain LPS is a virulence factor for parenterally-adapted serovar Enteritidis.

IMPACT/TECH TRANSFER D: It has been proposed that monitoring field isolates of serovar Enteritidis for their ability to produce high-molecular-weight O-chain LPS in quantity is an effective approach for identifying emergence of strains with enhanced virulence. The *wzz* mutants define the point at which O-chain switches between high and low molecular weight structures, which makes them important controls for conducting epidemiology studies.

PUBLICATIONS:

Gast, R. K. and P. S. Holt, 1998. Persistence of *Salmonella enteritidis* from one day of age until maturity in experimentally infected layer chickens. Poultry Sci. 77:1759-1762.

Gast, Richard K. 1999. Applying experimental infection models to understand thepathogenesis, detection, and control of *Salmonella enteritidis* in poultry. *In: Salmonella enterica* serovar Enteritidis in Humans and Animals: Epidemiology, Pathogenesis, and Control, A. M. Saeed, R. K. Gast, M. E. Potter, and P. G. Wall, eds., pp. 233-243. Iowa State University Press, Ames, IA.

Gast, Richard K., B. W. Mitchell, and P. S. Holt, 1999. Application of negative air ionization for reducing experimental airborne transmission of *Salmonella enteritidis* to chicks. Poultry Sci. 78:57-61.

Guard-Petter, J., C.T. Parker, K. Asokan, and R.W. Carlson. 1999. Clinical and veterinary isolates of *Salmonella enterica* serovar Enteritidis defective in lipopolysaccharide O-chain polymerization. Appl. Environ.l Microbiol. 65:2195-2201.

Guard-Petter, J. 1999. Phage typing and outer membrane characteristics of virulent *Salmonella* Enteritidis. *In Salmonella enterica* ser. Enteritidis in Humans and Animals, M. Saeed, ed., pp. 221-232. Iowa State Univ. Press.

Holt, P.S., B.W. Mitchell, K.H. Seo, and R.K. Gast. 1999. Use of air ionization for reducing airborne levels of *Salmonella enterica* serovar Enteritidis in a room containing infected caged layers. J. Appl. Poultry Sci. (in press)

Mitchell, B.W. 1998. Effect of negative air ionization on ambient particulates in a hatching cabinet. Agric. 14(5):551-555.

Waltman, W.D., R.K. Gast, and E.T. Mallinson, 1998. Salmonellosis. *In:* Isolation and Identification of Avian Pathogens, 4th edition, D. E. Swayne, et al (eds)., pp. 4-13. American Association of Avian Pathologists, Kennett Square, PA.

CONTROL OF SALMONELLA IN DOMESTIC ANIMALS

ARS Contact Persons:	CRIS NUMBER:	6612-42000-019
N.J. Stern, J.S. Bailey,	FSIS CODE:	I-82-27
N.A. Cox, S.E. Craven	CRIS TERM DATE:	September 2000

Russell Research Center
Poultry Microbiological Safety Research Unit
Athens, GA
Phone:706-546-3516
Fax: 706-546-3771
E-mail: nstern@ars.usda.gov

OBJECTIVE A: Identify and quantify sources and spread of *Salmonella* through all phases of broiler production.

PROGRESS A: A nationwide epidemiology project to identify sources of *Salmonella* was completed. All potential sources from the hatchery through processing were sampled at time of placement and at 2, 4, 6 weeks of grow-out and after transport and processing during the Spring, Summer, Winter and Fall from both high and a low production farms in Georgia, Alabama, Arkansas and California. In excess of 6000 samples were examined. Selected samples with salmonellae prevalence rates include: carcass rinses (6.08%), feed (2.73%), mice (52.5%), insects (4.6%), flies (26.67%), chick transport pads (51.76%), transport coops (6.15%). Appropriate isolates are being genetically typed to facilitate trace back from the processed carcass to the initial source.

IMPACT/TECH TRANSFER A: Meeting the Pathogen Reduction Final Rule *Salmonella* standards will likely require on farm interventions. The data generated in this project will greatly assist regulatory agencies, research institutions and the poultry industry in focusing research dollars and intervention strategies in the areas with the best probability of success in reduction of these pathogens. The National Chicken Council and the member companies who participated in this epidemiological study are working with this laboratory to set up a 12 to 15 million bird national on farm intervention study to address the areas of concern identified in this study. In addition, the quantitative data generated in this project is critically needed to facilitate the completion of accurate Risk Assessment Models.

OBJECTIVE B: Develop on-farm control procedures to prevent colonization of chickens and/or turkeys with *Salmonella.*

PROGRESS B: Under a Cooperative Research and Development Agreement with the Continental Grain Company, our patented mucosal competitive exclusion (MCE) culture has been successfully scaled up for commercial production by one of the largest fermentation companies in the world, CHR Hansen of Milwaukee, WI. CHR Hansen is in the final stages of approval for the

manufacturing chemistry section of the NADA. All other sections of the NADA have been signed off on by FDA In addition, Continental Grain has received regulatory approval to sell MSC in Brazil and Japan. The largest chicken company in Japan has purchased 2 million doses of MSC and has begun commercial application of the product. We have also shown in floor pen trials that litter treatments with aluminum sulfate or sodium bisulfate can reduce *Salmonella* carriage in the ceca and on the external surface of in chickens on the farm prior to processing.

IMPACT/TECH TRANSFER B: Competitive exclusion, yeast and litter treatments each offers the possibility of being able to significantly reduce the on-farm colonization of chickens with *Salmonella* thus helping the poultry industry to meet specified *Salmonella* standards.

OBJECTIVE C: Identify the source and spread of *Clostridium perfringens* in poultry production.

PROGRESS C: The first comprehensive study of *Clostridium perfringens* contamination in poultry production was conducted. Samples from two farms for each of two integrators were collected periodically during the grow-out cycle for broiler chickens in each of four seasons. Of the total 4568 farm samples collected, the incidence of *C. perfringens* contamination on selected sample types include: chick transport pads (31%), chicken fecal droppings (24%), feed (17%), wall and fan swabs (50%), flies (43%), insects (22%), mice (33%), transport coops (43%), processing scald water (61%). There appeared to be a positive correlation between levels of *C. perfringens* on the farm and in the processing plant. Genetic typing methods are being used to facilitate tracing back from the processed carcass to the initial source.

IMPACT/TECH TRANSFER C: A paucity of information is available about the incidence or sources of *C. perfringens* in poultry production. This information will allow the most effective use of resources to reduce this pathogen in poultry production and processing. Developing a cost-effective method to reduce *C. perfringens* may also provide an alternative to the use of growth-promoting antibiotics for control of this organism.

OBJECTIVE D: Determining the prevalence of salmonellae in primary broiler breeders.

PROGRESS D: Eggshell fragments, paper pads from chick boxes and fluff samples were obtained from three commercial primary breeder hatcheries and analyzed for the presence and level of salmonellae with identical laboratory methods used in 1991. Overall, 29 of 180 samples (16.1%) from the three hatcheries were salmonellae positive compared to 11.1% positive in 1991. From an enumeration standpoint, salmonellae levels were significantly reduced in 1998 where less than 4% of the positive samples had high levels of salmonellae compared to 64% of the 1991 samples with high levels.

IMPACT/TECH TRANSFER D: Both primary broiler breeders and broiler hatcheries present critical control points in the prevention of salmonellae contamination during commercial poultry production. This data demonstrates to the poultry industry that they must continue to diligently work to reduce salmonellae at this critical control point.

PUBLICATIONS:

Berrang, J.E., J.F. Frank, R.J. Buhr, J.S. Bailey and N.A. Cox, 1999. Eggshell membrane structure and penetration by *Salmonella typhimurium*. J. Food Prot. 62:73-76.

Chen, M., N. J. Stern, J. S. Bailey, and N. A. Cox. 1998. Optimum method to treat broiler chicks with mucosal competitive exclusion flora for control of Salmonellae. J. Appl. Poultry Research. (in press)

Cox, N.A., J.S. Bailey and M.E. Berrang, 1998. Bactericidal treatment of hatching eggs I. Chemical immersion treatments and *Salmonella*. J. Appl. Poultry Res. 7: 347-350.

Cox, N.A., M.E. Berrang, R.J. Buhr and J.S. Bailey, 1999. Bactericidal treatment of hatching eggs II. Use of chemical disinfectants with vacuum to reduce *Salmonella* on hatching eggs. J. Appl. Poultry Res. (in press).

Craven, S.E., N.J. Stern, N.A. Cox, J.S. Bailey, and M. Berrang. 1999. Cecal carriage of *Clostridium perfringens* in broiler chickens given Mucosal Starter Culture™. Avian Dis. (in press)

Fedorka-Cray, P., J.S. Bailey, N.J. Stern, N.A. Cox, S. Ladley and M. Musgrove. 1999. Mucosal competitive exclusion to reduce *Salmonella* in swine. J. Food Prot. (in press)

PROCEEDINGS/ABSTRACTS:

Bailey, J.S. 1999. Food safety epidemiology. Proc. Clemson Food Safety Symposium, April, 1999. pp 7-8.

Bailey, J.S.. 1998. Reduction of bacterial contamination of poultry products. Proc. Cornell Nutrition Conference. pp 85-91.

Bailey, J.S., N.A. Cox and N.J. Stern, 1999. Chlorination of chill tanks reducessalmonellae on processed broiler carcasses. Proc.86th Annual Meeting International Association of Milk Food and Environmental Sanitarians, pp. 63-64.

Berrang, M.E. and N.A. Cox, 1998. Hatchery Sanitation and Disinfection. Proc. of Georgia Poultry Conference. pp 40-44.

Cox, N.A. 1998. Incidence and impact of salmonellae in broiler hatcheries. Proc. International Symposium on Foodborne Salmonellae in poultry. Pp 88-92.

Cox, N.A. 1998. Salmonellae transmission in poultry. Proc. 1998 Georgia International Poultry Course. pp 80-87.

Cox, N.A., J.S. Bailey and N.J. Stern, 1999. Use of Mucosal Starter Culture™ to reduce *Salmonella* during broiler production. Proc. 1999 Midwest Poultry Federation, pp. 85-89.

Cox, N.A., J.S. Bailey, N.J. Stern and M.E. Berrang, 1998. Practical impact of competitive exclusion products on health. Proc. 25th Annual Carolina Poultry Nutrition Conference, Aquaculture Nutrition Symposium, and Soybean Meal Symposium, pp. 114-119.

Cox, N.A., J.S. Bailey, P.J. Fedorka-Cray, N.J. Stern and M.E. Berrang, 1998. Mucosal Starter Culture™ treatment to significantly reduce salmonellae in processed broiler carcasses and swine. Proc. 1998 World Health Organization Consultation on Vaccination and Competitive Exclusion against Salmonellae Infections in Animals. Oct 5-8, 1998., pp 1-16.

Cox, N.A., N.J. Stern and J.S. Bailey, 1999. Sources of *Salmonella* and *Campylobacter* in poultry and methods to intervene. Proc. 52nd Annual Reciprocal Meats Conference. pp 5-7.

Craven, S.E., N.J. Stern, P. Fedorka-Cray, J.S Bailey, R.J. Meinersmann, E. Line, and N.A. Cox. 1999. Epidemiology of *Clostridium perfringens* in the production and processing of broiler chickens. Proceedings of the 17th International Symposium of the International Committee on Food Microbiology and Hygiene, Veldhoven, The Netherlands., September, 1999.

Fedorka-Cray, P. and J.S. Bailey. 1999. Bugs and biosecurity: Is there room for both? Proc. 17th Annual Meeting American College of veterinary Internal Medicine, pp. 156-157.

PATENTS:

Report No. 0149.99 "Detection of *Salmonella typhimurium* DT104 Based on the Gene, *ST-flo*, which Confers Resistance to Florfenicol and Chloramphenicol" Currently, detection *of S. typhimurium* DT104 relies upon a battery of tradition microbial tests that typically take several weeks to complete. The discovery of *St-flo*, a gene that is unique to multiple drug resistant *S. typhimurium*, allowed for the design of primers, that when used in a PCR reaction, allow for the rapid, inexpensive, and presumptive identification of *S. typhimurium* DT104.

EPIDEMIOLOGY AND CONTROL OF *SALMONELLA*

ARS Contact Persons: **S.R. Bolin, T.J. Stabel, S.A. Carlson, S. Hurd**

CRIS NUMBER: 3625-32420-003
FSIS CODE: N1AM01
CRIS TERM. DATE: June 2002

National Animal Disease Center
Enteric Diseases and Food Safety Research Unit
Ames, IA
Phone: 515-663-7244
Fax: 515-663-7458
E-mail: sbolin@nadc.ars.usda.gov

OBJECTIVE A: Determine the epidemiologic factors that may be subjected to intervention measures for control of *Salmonella* in swine.

PROGRESS A: Developed computer simulation models to determine the effect of intermittent pathogen shedding on accurate estimating of population prevalence. Results suggest that simple random sampling protocols will underestimate the true prevalence. Field experiments are underway to measure the effect of lairage, mixing, and transportation on pathogen presence in pig feces and edible lymph nodes. In addition, the correlation between animal visceral contamination and carcass and meat contamination with *Salmonella* is also being studied.

IMPACT/TECH TRANSFER A: Sampling information will be important to researchers and federal agencies interested in quantitative data regarding the prevalence of *Salmonella* in the field and in individual animals. Field experiments will identify potential critical control points for effective control of *Salmonella* from farm to slaughter plant. As a result, producers, slaughter plants, regulatory agencies, and eventually the consumer will benefit from improved management practices. Results have been presented to commodity groups and other professionals at scientific meetings.

OBJECTIVE B: Define the immune response associated with acute and chronic *Salmonella* infection of swine.

PROGRESS B: Developed a simple, rapid two-color antibody phenotyping assay for porcine whole blood to identify common cell surface markers. Using flow cytometry, this assay allows reproducible and accurate sampling of a large number of pigs in < 2 hours. The effect of salmonellosis on blood cells and localized cell populations within tissues will be studied in large experimental and field trails.

IMPACT/TECH TRANSFER B: A rapid diagnostic test for blood cell abnormalities is a potential outcome of this new technique benefiting the field of veterinary medicine and eventually the consumers because of healthier swine and pork free of *Salmonella* that could cause human disease.

OBJECTIVE C: Characterize the epidemiology, transmission and nature of antibiotic resistance of *Salmonella typhimurium* DT104.

PROGRESS C: Developed DNA-based tests for multiresistant *Salmonella typhimurium* DT104 and other related organisms such as U302, DT193, and DT208. Identified and characterized a rare phenomenon in which the use of ineffective penicillin-like antibiotics can exacerbate the antibiotic resistance profile of multiresistant *S. typhimurium* DT104. Studied the invasive properties of multiresistant and nonresistant *S. typhimurium* DT104 *in vitro* and found that the multiresistant variety is no more invasive than the nonresistant variety and thus not a hyperinvasive superpathogen. Tested the hypothesis that *Salmonella* initiate disease in swine by attacking and damaging specialized cells in the intestine called M-cells. Using scanning electron microscopy we are currently studying the *in vivo* disease process associated with *S. typhimurium* DT104.

IMPACT/TECH TRANSFER C: DNA-based tests will allow rapid detection of DT104 contaminants in food and the environment. This test has been transferred to an ARS scientist for development as a detection method for food and fecal *S. typhimurium* DT104 contamination. This assay will benefit producers, packing plants, regulatory agencies, and ultimately, the consumer. Information regarding the use of ineffective penicillin-like antibiotics will provide important insight for the proper use of antibiotics on the farm. Information on the invasive potential of multiresistant *S. typhimurium* DT104 has been lacking and these new scientific data will help build a sound basis for policy decisions and public education. Identification of novel mechanisms in *S. typhimurium* DT104-host interaction and pathogenesis will allow the development of more effective vaccines.

PUBLICATIONS:

Carlson, S.A., L.F. Bolton, C.E. Briggs, H.S. Hurd, V.K. Sharma, P.J. Fedorka-Cray and B.D. Jones. 1999. Detection of multiresistant *Salmonella typhimurium* DT104 using multiplex and fluorogenic PCR. Mol. Cell. Probes 13:213-222.

ABSTRACTS:

Hurd, H.S., T.J. Stabel and S. Carlson. 1999. Sensitivity of various fecal sample collections techniques for detection of *Salmonella typhimurium* in finish hogs. 3rd Int. Symp. Epidemiol. Control of *Salmonella* in Pork Abstr., Washington, D.C., 3:63-64.

Hurd, H.S., W.D. Schlosser and E.D. Ebel. 1999. The effect of intermittent shedding on prevalence estimation in populations. 3rd Int. Symp. Epidemiol. Control of *Salmonella* in Pork Abstr., Washington, D.C., 3:57-62.

ANTIMICROBIAL RESISTANCE RESEARCH

ARS Contact Persons:
W. Barton
P.J. Fedorka-Cray

CRIS NUMBER: 6612-42000-028
FSIS CODE:
CRIS TERM. DATE: June 2000

Richard Russell Agricultural Research Center
Antimicrobial Resistance Research Unit
Athens, GA
Phone: 706-546-3305
Fax: 706-546-3066
E-mail: pcray@ars.usda.gov

OBJECTIVE A: To define mechanisms associated with the development of resistance to antimicrobials

PROGRESS A: A project has been initiated to define the insertion sites for resistance genes in *Salmonella typhimurium* DT104. Comparison and optimization of antimicrobial testing methodologies in order to accurately assess the degree of resistance in *Campylobacter* species is also underway.

IMPACT/TECH TRANSFER A: The DT104 work is a continuation of progress based on the identification of the *flo*$_{st}$ gene as an indicator for DT104. A CRADA was established with the Animal Health Institute to compare the antimicrobial testing methodologies. A recommendation to the National Committee on Clinical Laboratory Standards (NCCLS) which governs national testing standards for antimicrobial resistance will be made for adoption of the method.

OBJECTIVE B: To define the transfer of resistance between zoonotic pathogens and commensals and the effect resistance may have on the virulence or pathogenesis of the disease

PROGRESS B: The pathogenesis/virulence of DT104 was assessed in broiler chicks. We have determined that the level of colonization is increased in DT104 infected chicks when compared to chicks exposed to a strain of DT104 which is pan-sensitive. Current studies are underway to assess the rate at which resistance to fluoroquinolones develops in DT104 following treatment.

IMPACT/TECH TRANSFER B: This information is critical to understanding the effect resistance has on the pathogenesis of the disease. Assessment of the rate resistance develops following treatment under conventional rearing conditions will enable mitigation procedures to be developed. We are currently working with a pharmaceutical company in this phase of the study.

OBJECTIVE C: To develop rapid tests to detect antibiotic resistance.

PROGRESS C: We have determined that the resistance to florfenicol and chloramphenicol are conferred by the gene *flo*$_{st}$. Presumptive identification of DT104 can be made rapidly based on the presence of the *flo*$_{st}$ gene or its resulting phenotype.

IMPACT/TECH TRANSFER C: Two companies have expressed an interest in the technology and we are currently opening a dialogue with them. This information will allow for the development of a rapid test kit which will enable identification of DT104 in a more timely manner.

OBJECTIVE D: To monitor for the development/presence of resistance as part of the National Animal Health Monitoring System (NARMS)

PROGRESS D: Testing for the veterinary arm of the National Antimicrobial Resistance Monitoring Program (NARMS) is conducted in this laboratory. The NARMS is an interagency endeavor involving the USDA-ARS, USDA-APHIS, USDA-FSIS, FDA-CVM, and CDC. In 1998, there were 3318 *Salmonella* isolates overall of veterinary origin that were tested. These isolates represented a broad range of species and came from diagnostic laboratories (including 3 sentinel sites), healthy animals on farm, and raw product collected as part of the Hazard Analysis Critical Control Point (HACCP) rule. Additionally, the program expanded to include testing of *Campylobacter* and *E. coli* isolates.

IMPACT/TECH TRANSFER D: This project will facilitate transfer of information to regulatory agencies, research institutions, commodity groups, pharmaceutical industries and the veterinary and medical community. Analysis of this information will facilitate prudent and judicious use of antimicrobics and drive the research necessary to understand the mechanisms of resistance and develop alternatives to the use of antimicrobics.

OBJECTIVE E: To determine the environmental impact of resistance development and develop intervention strategies to mitigate the development/transfer/spread of resistance

PROGRESS E: A multi-state epidemiologic study was conducted in collaboration with investigators at UGA to determine the prevalence of DT104 in ground beef. A prevalence estimate of resistance in *Salmonella* and *Campylobacter* in broilers was also assessed. Currently, in collaboration with an investigator at UGA, we are studying the prevalence of *Salmonella*, *Campylobacter*, *E.coli* and enterococci in swine raised under three different antimcrobial use regimes and ascertaining the risk factors associated with resistance.

IMPACT/TECH TRANSFER E: Information from these studies are being transferred to commodity and consumer groups, pharmaceutical companies, and government agencies. A grant has been secured by the UGA swine collaborator from the NPPC who will use this information to make recommendations to minimize resistance to their producers.

PUBLICATIONS:

Akkina, J.E., A.T. Hogue, F.J. Angulo, R. Johnson, K.E. Petersen, P.K. Saini, P.J. Fedorka-Cray, and W.D. Schlosser. 1999. Epidemiologic aspects, control, and importance of multiple-drug resistant *Salmonella typhimurium* DT104 in the United States. J. Am. Vet. Med. Assoc. 214:790-798.

Apley, M.D., S.A. Brown, P.J. Fedorka-Cray, S. Ferenc, J.K. House, J.E. Riviere, L.B. Rice, C. Thornsberry, and J. Waddell. 1998. AAVPT Task Force Report: Role of veterinary therapeutics in bacterial resistance development: animal and public health perspectives. J. Am. Vet. Med. Assoc. 212:1209-1213.

Bolton, L.F., L.C. Kelley, M.D. Lee, P.J. Fedorka-Cray, and J.J. Maurer. 1999. Detection of multidrug-resistant *Salmonella enterica* serotype *typhimurium* DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J. Clin. Microbiol. 37:1348-1351.

Carlson, S.A., L.F. Bolton, C.E. Briggs, H.S. Hurd, V.K. Sharma, P.J. Fedorka-Cray, and B.D. Jones. 1999. Detection of multiresistant *Salmonella typhimurium* DT104 using multiplex and fluorogenic PCR. Mol. Cell. Probes 13:213-222.

Fedorka-Cray, P.J., D.A. Dargatz, L.A. Thomas, and J.T. Gray. 1998. Survey of *Salmonella* in feedlot cattle. J. Food Protect. 61:525-530.

Fedorka-Cray, P.J., D.A Dargatz, S.J. Wells, N.E. Wineland, M.A. Miller, L. Tollefson., and K.E. Petersen. 1998. Impact of antimicrobic use in veterinary medicine. J. Am. Vet. Med. Assoc. 213:1739-1741

Smith, D.R., P.J. Fedorka-Cray, R. Mohan, K.V. Brock, T.E. Wittum, P.S. Morley, K.H. Hoblet, and L.J. Saif. 1998. Evaluation of cow-level risk factors for the development of winter dysentery in dairy cattle. Am. J. Vet. Res. 59:986-993.

Smith, D.R., P.J. Fedorka-Cray, R. Mohan, K.V. Brock, T.E. Wittum, P.S. Morley, K.H. Hoblet, and L.J. Saif. 1998. Epidemiologic herd-level assessment of causative agents and risk factors for winter dysentery in dairy cattle. Am. J. Vet. Res. 59:994-1001.

Tollefson, L., F.J. Angulo, and P.J. Fedorka-Cray. 1998. National surveillance for antibiotic resistance in zoonotic enteric pathogens. In Veterinary Clinics of North America, pp. 141-150. W.B. Saunders, Co., Philadelphia, PA.

Tollefson, L., P.J. Fedorka-Cray, and F.J. Angulo. 1998. Public health aspects ofantibiotic resistance monitoring in the USA. Acta Scandinavica supplement. (in press)

PROCEEDINGS/ABSTRACTS:

Blaha, T., A.R. Carlson, and P.J. Fedorka-Cray. 1999. Investigations into the infection contamination infection cycle of zoonotic *Salmonella* on swine farms: serovar and sensitivity patterns of *Salmonella* isolates from animals and environments from selected Minnesota swine farms. 3rd International Symp. *Salmonella* in Swine, Washington, DC, August 5-7, 1999, pp.242-244.

Blaha, T. and P.J. Fedorka-Cray. 1999. The association of antimicrobial resistance patterns and reported usage of antimicrobials in commercial growing pig production. 3rd International Symp. *Salmonella* in Swine, Washington, DC, August 5-7, 1999, pp.240-241.

Bush, E.J., B. Wagner, and P.J. Fedorka-Cray. 1999. Risk factors associated with shedding of *Salmonella* by US finishing hogs. 3rd International Symp. *Salmonella* in Swine, Washington, DC, August 5-7, 1999, pp.106-108.

Cox, N.A., J.S. Bailey, P.J. Fedorka-Cray, N.J. Stern, and M.E. Berrang. 1999. Starter culture treatment to significantly reduce salmonellae in processed broiler carcasses and swine. WHO Consultation on Vaccination and Competitive Exclusion against *Salmonella* Infections in Animals, Jena, Germany, October 4-8, 1998

Dargatz, D.A., S.J. Wells, P.J. Fedorka-Cray, and J. Akkina. 1999. The veterinarian's role in diagnosis, treatment, and prevention of *Salmonella typhimurium* DT104. American Association of Bovine Practitioners Annual Meeting.

Davies, P.R., J.A. Funk, M.G. Nichols, J.M. O'Carroll, P.K. Turkson, W.A. Gebreyes, and P.J. Fedorka-Cray. 1999. Effects of some methodologic factors on detection of *Salmonella* in swine feces. 3rd International Symp. *Salmonella* in Swine, Washington, DC, August 5-7, 1999, pp.30-32.

Fedorka-Cray, P.J., D.A. Dargatz, M. Miller, and L. Tollefson. 1998. Antimicrobial susceptibility patterns of *Salmonella* isolates of veterinary origin. AAVPT Symp. Human Med. Impact of Food Animal Infect. Dis. And Their Treatment, January 19-21, College Park, MD, p.24.

Fedorka-Cray, P.J., M. Miller, L. Tollefson, and D.A. Dargatz. 1998. Development of resistance in *Salmonella* isolates of veterinary origin. Proc. American Assoc. Swine Prac., March 7-10, Des Moines, Iowa, pp.173-176.

Fedorka-Cray, P.J., D.A. Dargatz, M. Miller, and L. Tollefson. 1998. Antimicrobial Susceptibility Monitoring. AVMA Symposium on Food Safety Issues, July 25, Baltimore, MD, in press.

Fedorka-Cray, P.J., M. Miller, D.A. Dargatz, and L. Tollefson. 1998. Antimicrobial resistance patterns of *Salmonella* isolates of veterinary origin. Poultry Sci. Assoc., August 2-5, State College, PA.

Fedorka-Cray, P.J., K.E. Petersen, D.A. Dargatz, L. Tollefson, N.E. Wineland, M. Headrick, K. Hollinger, and K. Ferris. 1999. National Antimicrobial Resistance Monitoring System: Results for swine. 3rd International Symp. *Salmonella* in Swine, Washington, DC, August 5-7, 1999, pp.248-249.

Fedorka-Cray, P.J., P.B. Bahnson, and S.R.Ladely. 1999. Antimicrobial resistance patterns of *Salmonella* isolates collected from slaughter age pigs. 3rd International Symp. *Salmonella* in Swine, Washington, DC, August 5-7, 1999, pp.245-247.

Gray, J.T. and P.J. Fedorka-Cray. 1999. Detection of swine exposed to *Salmonella* species. 3rd International Symp. *Salmonella* in Swine, Washington, DC, August 5-7, 1999, pp.46-50.

Reeves, D.E., P.J. Fedorka-Cray, J.S. Bailey, N.J. Stern, N.A. Cox, S.R. Ladely, and W. Pullen. 1999. Mucosal competitive exclusion to reduce *Salmonella* in swine. Amer. Soc. An. Sci., 91st Annu. Meet., Indianapolis, IN. J. An. Sci. 77(S1):128-129.

Tollefson, L., M. Miller, P.J. Fedorka-Cray, F.J. Angulo, and S.F. Sundlof. 1998. FDA/CDC/USDA antibiotic resistance monitoring program. AAVPT Symp. Human Med. Impact of Food Animal Infect. Dis. And Their Treatment, January 19-21, College Park, MD. (in press)

Tollefson, L., P.J. Fedorka-Cray, N. Marino, and F. Angulo. 1999. US National Entimicrobial Resistance Monitoring System for Enteric Bacteria. WHO Meeting, Geneva, Switzerland, March 31-April 1, 1999.

Tollefson, L. F.J. Angulo, and P.J. Fedorka-Cray. 1999. Report on the informal information meting on antimicrobial resistance surveillance in foodborne pathogens. WHO Meeting, Geneva, Switzerland, March 31-April 1, 1999.

Traub-Dargatz, J.L., L.P. Garber, P.J. Fedorka-Cray, S.R. Ladely, K. Ferris, and D.A. Miller. 1999. National animal health monitoring system (NAHMS) equine '98 study results *Salmonella* spp. fecal shedding in the US horse population and presence of *Salmonella* spp. in grain sources on equine operations 1998-1999. American College Veterinary Internal Medicine Annual Meeting, June 10-12, Chicago, IL.

DEVELOPMENT OF COST EFFECTIVE MEANS TO PREVENT AND CONTROL SALMONELLOSIS IN POULTRY

ARS Contact Persons:	CRIS NUMBER	6202-42000-011
D.E. Corrier, L.H. Stanker,	FSIS CODE	C1AM01
J.A. Byrd, M.E. Hume,	CRIS TERM. DATE	September 2002
L.F. Kubena, R.L. Ziprin		

Food and Feed Safety Research Unit
Southern Plains Agricultural Research Center
College Station, TX
Phone: 409-260-9484
Fax: 409-260-9332
E-mail: corrier@ffsru.tamu.edu

OBJECTIVE A: Develop effective means to prevent/control *Salmonella* colonization of broiler chickens during growout to market age.

PROGRESS A: Final FDA pivotal field studies to evaluate the efficacy of patented competitive exclusion culture in commercial broiler flock were completed. Our CRADA partner, BioScience Division of Milk Specialties Company, Dundee, IL, completed the final report and submitted it for FDA approval and registration.

IMPACT/TECH TRANSFER A: In March 1998, the FDA approved the use of Milk Specialties' produced CF3 culture under the tradename PREEMPT™ for use in commercially produced broiler chickens. This was the first competitive exclusion culture to receive FDA approval for use in commercial poultry flocks.

OBJECTIVE B: Evaluate the chicken crop as a source of *Salmonella* and *Campylobacter* contamination of poultry slaughter plants.

PROGRESS B: Feed is withdrawn from broiler chickens 6 to 12 hours before transport to the processing plant. During feed withdrawal, birds increasingly peck at fecal droppings and litter on the rearing house floor. The fecal droppings and litter may contain pathogenic bacteria that contaminate the carcass in the processing plant. Our laboratory demonstrated that both *Salmonella* and *Campylobacter* contamination of the crop increases dramatically during this feed withdrawal period. Additionally, we found that feed withdrawal resulted in a decrease in lactic acid concentration in the crop, accompanied by an increase in crop pH, which may account for the increase in crop contamination. Furthermore, conditions found in the crop after feed withdrawal were found to increase the virulence of salmonellae. These results strongly suggest that preslaughter feed withdrawal is a critical control point for reducing potential *Salmonella* and *Campylobacter* contamination of processed poultry meat products.

IMPACT/TECH TRANSFER B: Experimental data from our laboratory suggest that organic acids (acetic, lactic, and formic) provided to broilers during an 8 hour feed withdrawal will maintain crop pH and will provide an unfavorable environment for pathogenic bacteria. Presently, 2 commercial field trials have been performed with 2 large broiler production companies in Texas and Mississippi to use this cost-efficient strategy to reduce *Salmonella* and *Campylobacter* contamination on commercial broiler farms. A third trial will be performed during the upcoming year. Preliminary data from the field trials suggest a 50% reduction in *Salmonella* and a 20% reduction in *Campylobacter* in pre-chill carcass rinses. Presently, 2 commercial broiler producers are evaluating lactic acid as a means of reducing pathogenic bacteria contamination in pre-harvest broilers which may translate into reduced contamination of poultry carcasses within the poultry processing plants.

OBJECTIVE C: Evaluate a fluorescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants.

PROGRESS C: Recently, our laboratory demonstrated that both *Salmonella* and *Campylobacter* contamination of the crop increases dramatically during this feed withdrawal period in broilers. As these broilers travel through the initial stages of slaughter, carcass contamination increases with the rupture or leakage of the gastrointestinal tract. Therefore, our laboratory developed a marker to investigate the possible leakage of the upper gastrointestinal tract. Presently, we evaluated the marker at 5 commercial broiler processing plants and reported that over 60% of the carcasses were contaminated with the marker after feather removal. Carcasses had a marker contamination rate of greater than 90% after evisceration and 63% of the carcasses were contaminated after the final wash before entering the chill tank.

IMPACT/TECH TRANSFER C: Our research approach may well lead to the selection of new processing equipment which may reduce contamination, assisting adjusting existing equipment, and the marker may be utilized as a teaching tool to educate poultry processing personnel on the importance of personal and carcass hygiene.

OBJECTIVE D: Evaluate the effect of feed withdrawal during forced molt on *Salmonella* survival in the crops of leghorn hens and investigate the mechanism(s) by which feed withdrawal during forced molt reduces resistance to *Salmonella.*

PROGRESS D: Forced molt induced by feed withdrawal is used to stimulate egg laying in aging flocks of hens. Feed removal during forced molt decreases the resistance of hens to *S. enteritidis* resulting in severe infections and organ invasion. The mechanism by which feed withdrawal decreases resistance to infection is unknown. We recently reported that preslaughter feed withdrawal in market age broilers results in increased *Salmonella* crop contamination and that crop contamination is directly associated with decreased concentrations of crop lactic acid and increased crop pH. Similarly, we found that during forced molt, increased *S. enteritidis* crop contamination is also associated with a decrease in crop lactic acid and an increase in pH. The results suggest that

a common mechanism may account for the increased incidence of crop contamination during preslaughter feed withdrawal in broiler flocks and forced molted aging hens.

IMPACT/TECH TRANSFER D: Increased survival of *Salmonella* in the crops of broilers during preslaughter field withdrawal and leghorn hens during forced molt is clearly associated with decreased acidity of the crop. Provision of acids in the drinking water of broilers and layer hens during periods of feed withdrawal reestablishes the acidity of the crop and reduces *Salmonella* crop contamination.

OBJECTIVE E: To identify and characterize factors affecting attachment and colonization of the chicken gastrointestinal tract by *Campylobacter*.

PROGRESS E: In cooperation with Washington State Univeristy, we studied two genes involved in colonization of the gastrointestinal tract of chickens by *Campylobacter jejuni*. These genes are *pldA*, which encodes for a phospholipase, and *ciaB*, which is involved in cell invasion, *in vitro*. We found that *Campylobacter jejuni* strains carrying mutations in either locus are poor colonizers, and are avirulent when given either *in ovo*, or on day-of-hatch. Further, we found that these mutants and two others, *cadF* and *dnaJ*, were not able to induce a protective immunity against the parent strain of *Campylobacter jejuni*.

IMPACT/TRANSFER E: Though still not successful, our research approach may lead to the development of a vaccine against colonization of the chicken gastrointestinal tract by *Campylobacter*. A successful vaccine would have a significant impact on food safety. In addition, the knowledge gained by our studies of the molecular-genetic basis for colonization should strengthen the overall understanding of natural processes of colonization by *Campylobacter jejuni* in chickens.

OBJECTIVE F: Evaluate the effects of specific dietary constituents and toxins on the efficacy of indigenous flora and Competitive Exclusion (CE) Cultures to protect against salmonellae colonization in broiler chicks.

PROGRESS F: Disruption of the native microflora or administered CE cultures by dietary components or additives could affect the concentrations of volatile fatty acids (VFA), perhaps making the chickens more susceptible to colonization by *Salmonella* and/or other enteropathogens. Studies were conducted using broiler chicks fed unmedicated diets (controls) or these unmedicated diets containing added tannic acid, fumonisin B1, and/or moniliformin from culture material. Feeding relatively high concentrations of any of these additions did not alter cecal propionic acid and/or total VFA. Previously, we showed a high concentration of T-2 toxin fed to chicks administered a CE culture were more susceptible to cecal colonization by *Salmonella typhimurium* than chicks fed a control diet or a lower concentration of T-2 toxin. These results indicate that diets contaminated with relatively high levels of toxins can affect VFA concentrations, and in some cases, the susceptibility to *Salmonella* colonization. Further

research in this area is critical for the successful implementation of an integrated multi-faceted salmonellae prevention and control program in poultry.

IMPACT/TECHNOLOGY TRANSFER F: This research has been presented formally to fellow scientists, poultry industry representatives, and allied industry representatives. Two abstracts have been published and a manuscript has been submitted to a refereed Journal for publication. There have also been several discussions in person and by telephone with interested parties.

PUBLICATIONS:

Barnhart, E.T., D.J. Caldwell, M.C. Crouch, J.A. Byrd, D.E. Corrier and B.M. Hargis. 1999. Effect of lactose administration in drinking water prior to and during feed withdrawal on *Salmonella* recovery from broiler crops and ceca. Poultry Sci. 78:211-214.

Barnhart, E.T., D.J. Caldwell, M.C. Crouch, J.A. Byrd, D.E. Corrier and B.M. Hargis. 1999. Evaluation of potential disinfectants for pre-slaughter broiler crop decontamination. Poultry Sci. 78:32-37.

Byrd, J.A., D.E. Corrier, M.E. Hume, R.H. Bailey, L.H. Stanker and B.M. Hargis. 1998. Effect of feed withdrawal on *Campylobacter* in the crops of market-age broiler chickens. Avian Dis. 42:802-806.

Byrd, J.A., J.R. DeLoach, D.E. Corrier, B.M. Hargis, D.J. Nisbet and L.H. Stanker. 1999. Evaluation of *Salmonella* serotype distributions from commercial broiler hatcheries and grower houses. Avian Dis. 43:39-47.

Corrier, D.E., J.A. Byrd, B.M. Hargis, M.E. Hume, R.H. Bailey and L.H. Stanker. 1999. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poultry Sci. 78:45-49.

Corrier, D.E., J.A. Byrd, B.M. Hargis, M.E. Hume, R.H. Bailey and L.H. Stanker. 1999. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. Avian Dis. 43:453-460.

Durant, J.A., D.E. Corrier, J.A. Byrd, L.H. Stanker and S.C. Ricke. 1999. Feed deprivation affects crop environment and modulates *Salmonella enteritidis* colonization and invasion of leghorn hens. Appl. Environ. Microbiol. 65(5):1915-1923.

Konkel, M. E., B. J. Kim, J. D. Klena, C. R. Young and R. Ziprin. 1998. Characterization of the thermal stress response of *Campylobacter jejuni*. Infect. Immun. 66:3666-3672.

Nisbet, D.J., G.I. Tellez, M.H. Kogut, D.E. Corrier, V.K. Lowry, M.L. Hernandez, J.R. DeLoach and L.H. Stanker. 1998. Effect of a commercial competitive exclusion culture (PREEMPT™) on mortality and horizontal transmission of *Salmonella gallinarum* in broiler chickens. Avian Dis. 42:651-656.

PROCEEDINGS/ABSTRACTS:

Byrd, J.A., D.E. Corrier, M.E. Hume, R.H. Bailey, B.M. Hargis, and L.H. Stanker. 1998. Effect of feed withdrawal on the incidence of *Salmonella* and *Campylobacter* in the crops and ceca of market age broiler chickens. Proceedings to International Symposium on Food-borne *Salmonella* in Poultry, pg. 299.

Byrd, J.A., B.M. Hargis, D.E. Corrier, R.L. Brewer, D.L. Caldwell, and L.H. Stanker. 1999. Fluorescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants. Poultry Sci. 78 (Suppl 1):124.

Byrd, J.A. 1999. Pre-harvest intervention strategies. Proceedings to 71st Northeastern Conference on Avian Diseases pg. 6-15.

Byrd, J.A., D.E. Corrier, D.J. Caldwell, R.H. Bailey, R.L. Brewer, L.H. Stanker, and B.M. Hargis. 1999. Effect of selected organic acids on the control of *Salmonella* in market-age broilers during feed withdrawal. Poultry Science 78 (Suppl 1): 85.

Byrd, J.A. 1999. Origin and relationship of *Campylobacter* and *Salmonella* contamination of poultry during processing. Poultry Sci. 78: (Suppl 1) 5.

Corrier, D.E., J.A. Byrd, B.M. Hargis, M.E. Hume, R.H. Bailey, and L.H. Stanker. 1998. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. Poultry Science 78 (suppl):95.

Crouch, M.C., D.Y. Caldwell, R.W. Moore, J.A. Byrd, D.E. Corrier, and B.M. Hargis. 1998. Isolation of a *Bacillus* from chicken cloaca with in vitro competitive exclusion properties for *Salmonella* and *Staphylococcus*. Poultry Science 77 (suppl):115.

Durant, J.A., D.E. Corrier, J.A. Byrd, L.F. Kubena, and S.C. Ricke. 1999. Effect of feed withdrawal during forced molt of leghorn hens on the crop environment and *Salmonella enteritidis* invasion and colonization. Poultry Sci. 78 (Suppl 1):99.

Hargis, B.M., D.J. Caldwell, J.A. Byrd, D.E. Corrier, and L.H. Stanker. 1998. Preharvest crop contamination and *Salmonella* recovery from broiler carcasses at processing. Proceedings from International Symposium on Food-borne *Salmonella* in Poultry, pg 220-231.

Kubena, L.F., R.H. Bailey, J.A. Byrd, C. Young, and D.E. Corrier. 1998. Effect of aflatoxins of cecal volatile fatty acids and susceptibility to *Salmonella typhimurium* colonization in broiler chicks. Poultry Sci. 77(suppl):115.

Kubena, L.F., J.A. Byrd, C. Young, and L.H. Stanker. 1999. Effect of tannic acid on cecal volatile fatty acids and susceptibility to *Salmonella typhimurium* colonization in broiler chicks. Poultry Sci. 78 (Suppl 1):100.

Kubena, L.F., J.A. Byrd, D.E. Corrier, L.H. Stanker, and G.E. Rottinghaus. 1999. Effect of feeding fumonisin B1 and moniliformin present in culture material on cecal volatile fatty acids and susceptibility to *Salmonella typhimurium* colonization in broiler chicks. Poultry Sci. 78 (Suppl 1): 84.

Young, C. R., R.L. Ziprin, M.E. Hume, L.H. Stanker, and M.E. Konkel. 1998. Dose response and organ invasion of day-of-hatch leghorn chicks by different isolates of *Campylobacter jejuni*. Poultry Sci 77(suppl 1):41.

Young, S.D., J.A. Byrd, D.Y. Caldwell, D.J. Caldwell, L.H. Stanker, and B.M. Hargis. 1999. Evaluation of upper gastrointestinal leakage on *Salmonella* carcass contamination during turkey processing. Poultry Sci. 78 (Suppl 1):83.

Young, S.D., M.B. Farnell, J.A. Byrd, D.Y. Caldwell, D.J. Caldwell, D.E. Corrier, L.H. Stanker, and B.M. Hargis. 1999. Evaluation of alternative sampling methods and critical control point determination for *Salmonella* at turkey processing. Poultry Sci. 78 (Suppl 1):100.

Ziprin, R. L., C.R. Young, M.E. Hume, L.H. Stanker, B.J. Kim, and M.E. Konkel. 1998. Inability of *Campylobacter jejuni* exhibiting defective environmental stress response to colonize the ceca of newly hatched chicks. Poultry Sci 77 (suppl 1): 28.

Ziprin, R.L., M.E. Konkel, M.E. Hume, L.H. Stanker, S.A. Gray, B.J. Kim, and C.R. Young. 1999. Effect of mutations in *Campylobacter jejuni* genes on cecal colonization and liver invasion, when given *in ovo* or on day-of-hatch. Poultry Sci. 78 (suppl 1):167.

CONTROL OF *SALMONELLA* AND *ESCHERICHIA COLI* O157:H7 IN LIVESTOCK DURING THE PREHARVEST PERIOD

ARS Contact Persons:
W.W. Laegreid, J.E. Keen, R.O. Elder, C.G. Chitko-McKown, D.B. Laster

CRIS NUMBER: 5438-42000-003
FSIS CODE: M1AM01
CRIS TERM. DATE: July 1999

Animal Health Research
U.S. Meat Animal Research Center
Clay Center, NE 68933
Phone: (402) 762-4180
Fax: (402) 762-4375
E-mail: laegreid@E-mail.marc.usda.gov

OBJECTIVE A: To develop diagnostic tests for foodborne pathogens in livestock.

PROGRESS A: Methodology for improved isolation and culture of enterohemorrhagic *E. coli* from fecal, hide and environmental samples has been developed and validated. This methodology has been shown to have very high sensitivity in detection of *E. coli* O157:H7 in field samples. Utilizing this methodology, prevalence of *E. coli* O157:H7 in cattle at various stages of the production cycle has been shown to be approximately 10-fold higher than previously described. Monoclonal antibodies (MAb) directed against enterohemorrhagic *E. coli* serotypes other than O157:H7 have been developed. MAb with specificity for O26, O111 and O103, serotypes implicated in foodborne illness in the U.S. and other countries around the world, have been developed and tested. Preliminary studies show that enterohemorrhagic *E. coli* of these O serotypes are present in the gastrointestinal tracts of cattle in the U.S.

IMPACT/TECH TRANSFER A: There has been great interest in this methodology among industry, research, academic, diagnostic and regulatory groups. Scientists from major meat packing companies, several universities, NVSL, NAHMS and other ARS researchers have been provided with the protocol and/or been trained in the use of this technique in our laboratories. Widespread use of culture techniques with greater sensitivity will significantly improve monitoring of infected livestock, evaluation of intervention strategies and detection of environmental contamination. As the recent outbreak of enterohemorrhagic *E. coli* O111 in Texas demonstrated, serotypes other than O157:H7 represent threats to the U.S. food supply. Serotypes O26, O111 and O103, among others, are relatively more common elsewhere in the world and our studies have shown them to be present in U.S. cattle. Use of MAb to these O antigen types in rapid diagnostic and screening tests will provide an estimate of the potential risk to the U.S. meat supply.

OBJECTIVE B: Characterize the preharvest epidemiology and ecology of foodborne pathogens in livestock.

PROGRESS B: Studies to determine the effects of management interventions on the prevalence of naturally occurring *E. coli* O157:H7 infection in feedlot cattle were performed. Effects of dietary shift, pen sanitation and transportation to slaughter were evaluated. Shifting to a hay-based diet from a standard grain-based feedlot ration significantly reduced the percentage of cattle with O157:H7 in their feces after seven days. Significant weight loss and collateral health effects were associated with switching to a hay diet. Simulated transport of slaughter weight cattle caused a similar reduction in percentage of cattle shedding *E. coli* O157:H7 in feces. Sanitation measures including complete washing of concrete floored pens three times a week and maintaining clean waterers had no significant effect on percentage of cattle shedding *E. coli* O157:H7 in feces.

IMPACT/TECH TRANSFER B: Reduction of numbers of cattle infected with *E. coli* O157:H7 should significantly reduce the risk of contamination of the U.S. meat supply. Methods to reduce numbers of infected cattle should be evaluated in naturally infected animals under production conditions to minimize costs of instituting ineffective methods and to estimate unintended outcomes such as adverse health effects. These studies found that two plausible control strategies were either ineffective (high levels of pen sanitation) or associated with significant adverse health and economic effects. Furthermore, the inadvertent beneficial effect of transport was demonstrated. These results have been communicated to cattle producer groups directly (NCBA) and through the lay press.

PUBLICATIONS:

Basaraba, R.J., A.N. Byerly, G.C. Stewart, D.A. Mosier, B.W. Fenwick, M.M. Chengappa and W.W. Laegreid 1998. Actin enhances the haemolytic activity of *Escherichia coli*. Microbiology 144:1845-1852.

Laegreid, W.W., R.O. Elder, and J.E. Keen. 1999. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. Epidemiol. Infect. (in press)

PROCEEDINGS/ABSTRACTS:

Elder, R.O. and J.E. Keen. 1999. Effects of pen cleaning and group vs. individual penning on fecal shedding of naturally-acquired enterohemorrhagic *Escherichia coli* (EHEC) O157 in beef feedlot cattle. 80th Conf. Res. Work. Anim. Dis., Chicago, IL, November 7-9, 1999.

Keen, J.E., G.A. Uhlich, and R.O. Elder. 1999. Effects of hay- and grain-based diets on fecal shedding of naturally-acquired enterohemorrhagic *Escherichia coli* (EHEC) O157 in beef feedlot cattle. 80th Conf. Res. Work. Anim. Dis., Chicago, IL, November 7-9, 1999.

Rivera, M. and J.E. Keen. 1999. Identification and detection of *Escherichia coli* O26 and O111 using monoclonal antibodies. Am. Soc. Microbiol. 99th Gen. Mtg., May 30-June 3, 1999. #PC450.

PREVENTION OF LOSSES FROM COLIBACILLOSIS AND *ESCHERICHIA COLI* O157:H7 IN CATTLE AND SWINE

ARS Contact Persons:
S. Bolin, T. Casey,
E. Nystrom, V. Sharma
M. Rasmussen

CRIS NUMBER: 3625-32420-001
FSIS CODE: N1AM02
CRIS TERM. DATE: February 2001

National Animal Disease Center
Enteric Diseases and Food Safety Research
Ames, IA
Phone: 515-663-7244
Fax: 515-663-7458
E-Mail: sbolin@nadc.ars.usda.gov

OBJECTIVE A: Identify site and mechanism of O157:H7 colonization in cattle and identify other virulence attributes of O157:H7 and other Shiga toxin-producing *E. coli*.

PROGRESS A: We have previously shown that neonatal calves, weaned calves, and colostrum-deprived neonatal piglets are susceptible to colonization and intestinal damage caused by *E. coli* O157:H7. In addition, neonatal calves and colostrum-deprived neonatal piglets, but not weaned calves, develop severe and sometimes fatal disease. We have shown that an *E. coli* O157:H7 surface molecule called intimin is required for colonization. This suggests that an immune response directed against intimin may prevent colonization and reduce shedding of *E. coli* O157:H7. We have found that Shiga toxin (Stx) is required for symptomatic disease but that Stx is not required for colonization in neonatal calves and colostrum-deprived neonatal piglets. Weaned calves inoculated with *E. coli* O157:H7 remain healthy but become colonized. Unlike in neonatal calves and piglets, both intimin and Stx are involved in the colonization of weaned calves.

We have extended these experimental *E. coli* O157:H7 infection studies to colostrum-fed (suckling) neonatal piglets as an initial step for determining if antibodies directed against intimin will reduce *E. coli* O157:H7 colonization and shedding (see Objective B). We compared the pathogenicity of *E. coli* O157:H7 in neonatal colostrum-fed (suckling) piglets with that in colostrum-deprived piglets to determine if suckling piglets can be used in passive immunization studies. We showed that *E. coli* O157:H7 colonized and damaged the intestines of suckling and colostrum-deprived piglets similarly, but caused a more severe systemic disease which occurred earlier and in more suckling piglets than colostrum-deprived piglets. Based on these results, we concluded that suckling piglets will be a useful animal model for the intimin vaccine studies (see Objective B) and for studying systemic disease caused by Stx-producing *E. coli*.

IMPACT/TECH TRANSFER A: The experimental *E. coli* O157:H7 infection models in neonatal and weaned calves and in colostrum-deprived and suckling neonatal piglets developed under this objective are useful for determining the role of specific virulence attributes of *E. coli* O157:H7.

They have been used to demonstrate that intimin is required for colonization in neonatal calves, weaned calves, colostrum-deprived piglets, and suckling piglets. We have also used these models to show that although Stx is required for causing symptomatic disease in neonatal piglets, it is not required for intestinal colonization and damage in either neonatal piglets or calves. However, Stx appears to have a role in colonization of weaned calves. These experimental *E. coli* O157:H7 infection models will be useful in determining the role of other *E. coli* O157:H7 virulence attributes, for determining the effectiveness of vaccines and other intervention strategies (see Objective B), and for studying Stx-induced systemic disease.

OBJECTIVE B: Identify methods to reduce shedding of O157:H7 in cattle.

PROGRESS B: The results in Objective A demonstrate that intimin and Stx are potential vaccine targets to reduce colonization and shedding of *E. coli* O157:H7 in cattle. Although our ultimate goal is to reduce *E. coli* O157:H7 infection in cattle, we need a surrogate animal model to test our hypothesis that antibodies against intimin can prevent *E. coli* O157:H7 colonization and disease. The results in Objective A show that suckling piglets are colonized, develop intestinal lesions and severe systemic disease following inoculation with *E. coli* O157:H7. We plan to vaccinate pregnant sows with intimin and experimentally challenge their suckling piglets with *E. coli* O157:H7 to test the efficacy of anti-intimin vaccines. In preparation for these vaccine experiments, we have conducted additional prerequisite experiments to determine the minimal infective dose of *E. coli* O157:H7 and to determine if an *E. coli* O157:H7 strain that does not produce Stx will colonize suckling piglets.

Large inoculum doses have been routinely used in the calf and neonatal pig models of *E. coli* O157:H7 infections. These doses contain 10^9 to 10^{10} bacteria and are much higher than the number of *E. coli* O157:H7 likely to cause natural infections. Using lower and more relevant inocula for experimental infection models will facilitate pathogenesis and intervention studies. We determined the minimum infection dose of *E. coli* O157:H7 for neonatal colostrum-deprived piglets by inoculating these piglets with doses of *E. coli* O157:H7 ranging from < 1 to 10^{10} bacteria. We found that as few as 1 to 10 bacteria, like the higher doses, colonized and caused intestinal lesions, and sometimes also caused systemic disease in these piglets by 2 days postinoculation. This demonstrates that the infectious dose of *E. coli* O157:H7 is very low in neonatal piglets, similar to the infectious dose for humans, and perhaps for cattle.

In other experiments, we found that an *E. coli* O157:H7 strain that does not produce Shiga toxin did not cause rapid and severe systemic disease in suckling piglets, but did colonize and damage the intestines of suckling piglets similarly to toxigenic *E. coli* O157:H7. These results confirmed that Shiga toxin is responsible for systemic disease in neonatal piglets, but is not required for intestinal colonization and damage, which we hypothesize will be prevented by antibodies to intimin. Therefore, for humane reasons, we will use the nontoxigenic *E. coli* O157:H7 for our initial vaccine studies.

INPACT/TECH TRANSFER B: The results in Objective B, together with those in Objective A, demonstrate that neonatal suckling piglets are a suitable model to determine if anti-intimin antibodies can prevent *E. coli* O157:H7 colonization. This is an important step in determining if vaccination with intimin will prevent or reduce *E. coli* O157:H7 shedding in cattle. The results in Objectives A and B are part of an ongoing collaboration with Dr. A. D. O'Brien (Uniformed Services University of the Health Sciences, Bethesda, MD) and Dr. C. Neal Stewart, Jr. (University of North Carolina, Greensboro,NC) and are part of a USDA grant (Project No. 3625-32420-001-03) to determine if antibodies to intimin will prevent *E. coli* O157:H7 transmission and shedding in cattle.

OBJECTIVE C: Develop rapid methods to identify and quantify *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* pathogenic for humans in tissues and fluids from cattle.

PROGRESS C: Methods for the rapid detection, identification, and characterization of *E. coli* O157:H7 are important for monitoring the safety of beef and dairy products, identifying cattle shedding *E. coli* O157:H7, and for other epidemiological and ecological studies. A semi-automated fluorogenic polymerase chain reaction (PCR) for the specific detection of *E. coli* O157:H7 was developed. The assay simultaneously detects DNA sequences for 3 different genes in *E. coli* O157:H7, differentiates between O157:H7 and other Shiga toxin-producing *E. coli*, and was useful for detecting small numbers of these bacteria in samples of beef and in cattle feces.

Sensitive and rapid methods and devices for the detection of feces on the carcasses of slaughtered food animals are needed to identify and prevent contamination, support the FSIS "Zero tolerance" for fecal contamination rule, and reduce the incidence of human foodborne diseases. In collaboration with researchers at Iowa State University, we have developed a patented method to specifically detect an intrinsic fluorescent molecule, present in feces, which was identified as a digestion product of chlorophyll. Based on this method, we designed and constructed a portable, hand-held, prototype device which specifically detected small amounts of fecal contamination on meat in near-real time. This fecal detection device, and other devices based on the same method, will be useful to the meat packing industry for detecting feces on carcasses and as a tool to prevent contamination and improve food safety.

IMPACT/TECH TRANSFER C: The fluorogenic PCR method for the detection and characterization of *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* is rapid and sensitive and will be useful for APHIS, FSIS, veterinary diagnostic laboratories, meat packing plants. We have transferred this technology, with detailed information for performing the assay, to eight separate organizations.

U.S. Patent 5,914,247 entitled "Method and system for detecting fecal and ingesta contamination on the carcasses of meat animals" was obtained by ARS and Iowa State University. ARS and Iowa State University have granted a license to eMerge Interactive (Sebastian, FL) for this technology. A CRADA has been established with this company, ARS, and Iowa State University to design and

test commercially useful devices based on this method. All of the partners anticipate that such fecal detection devices can be available to the slaughter industry in the next two to three years.

PUBLICATIONS:

Nystrom, E.A., B.T. Bosworth, A.D. O'Brien and H.W. Moon. 1999. Bovine infection with *Escherichia coli* O157:H7. pp. 51-57. *In: Escherichia coli* O157 in Farm Animals. C.S. Stewart and H.J. Flint (eds.) CABI Publishing, Wallingford, UK.

Sharma, V.K., E.A. Nystrom and T.A. Casey. 1999. Semi-automated fluorogenic PCR assays (Taqman) for rapid detection of *Escherichia coli* O157:H7 and other Shiga toxigenic *E. coli*. Mol. Cell. Probes 13:291-302.

ABSTRACTS:

Cornick, N., T.A. Casey, and H.W. Moon. 1999. Model for competitive colonization of sheep by *E. coli* O157:H7. 99th Annu. Meet. Am. Soc. Microbiol. 99:269.

Nystrom, E.A., J. Pohlenz, H.W. Moon and A.D. O'Brien. 1999. *Escherichia coli* O157:H7 cause severe systemic disease in neonatal suckling piglets. Virulence Mechanisms of Bacterial Pathogens Meeting, Ames, IA.

Sharma, V.K., E.A. Nystrom and T.A. Casey. 1999. Development of fluorogenic PCR assays for rapid detection of virulence genes of *Escherichia coli* O157:H7 and other Shiga toxigenic *E. coli*. Virulence Mechanisms of Bacterial Pathogens Meeting, Ames, IA.

PATENTS:

Casey, T.A., M.A. Rasmussen and J.W. Petrich. 1999. Method and system for detecting fecal and ingesta contamination on the carcasses of meat animals. Patent Number 5,914,247. Issued June 22, 1999.

CONTROL OF *CAMPYLOBACTER* JEJUNI IN POULTRY

ARS Contact Persons:	CRIS NUMBER:	6612-42000-018
N.J. Stern, J.E. Line	FSIS NUMBER	I-82-27
N.A. Cox	CRIS TERM. DATE:	February 2000

Russell Research Center
Poultry Microbiological Safety Research Unit
Athens, GA
Phone: 706/546-3516
Fax: 706/546-3771
E-mail: nstern@ars.usda.gov

OBJECTIVE A: Identify and quantify sources and spread of *Campylobacter* through all phases of broiler production leading to contamination of the processed carcass. Identify the most critical sources contributing to the transfer and spread of *Campylobacter* spp. in production to the fully processed poultry. Determine exposure to consumer using risk assessment data analysis.

PROGRESS A: A nationwide epidemiology project to identify sources of *Campylobacter* was completed. All potential sources from the hatchery through processing were sampled at time of placement and at 2, 4, 6 and 8 weeks of production and after transport and processing during the Spring, Summer, Winter and Fall from both high and a low production farms in Georgia, Alabama, Arkansas and California. Approximately 11,000 samples were examined. Selected samples with *Campylobacter* prevalence rates include: carcass rinses (29.11%), water cups (1.26%), mice (3.51%), insects (3.97%), fly strips (3.73%), chick transport pads (0 %), transport coops (67.50 %), boot swabs (7.22%), fan blade swabs (2.25 %) and fecal droppings at 8 weeks of production (96 %). Isolates are being genetically subtyped to facilitate trace back from the processed carcass to the initial sources.

IMPACT/TECH TRANSFER A: Meeting any new requirements for *Campylobacter* control will likely require on farm interventions. The data generated in this project will greatly assist regulatory agencies, research institutions and the poultry industry to focus intervention strategies with the best probability for success in reducing public exposure to *Campylobacter*. The National Chicken Council and the member companies who participated in this epidemiological study are working with the Poultry Microbiological Safety Research Unit to create a 12 to15 million treated bird national, on-farm intervention study to reduce consumer exposure. In addition, the quantitative data generated in this project is critically needed to facilitate accurate Risk Assessment Models.

OBJECTIVE B: Develop a sub-typing system to characterize *Campylobacter* and *Salmonella* populations to track through various habitats.

PROGRESS B: A defined *Campylobacter* library consisting of 137 isolates and a defined *Salmonella* library consisting of 100 isolates were analyzed using ribotyping, RAPD, PFGE, AFLP,

serotyping (*Salmonella* only), and DNA sequencing. Each sub-typing method was optimized for analysis of either *Campylobacter* or *Salmonella*. The subsequent evaluation of these subtyping methods is currently in progress. As subtyping methods are selected, analyses of the approximately 1700 *Campylobacter* samples will be undertaken.

IMPACT/TECHNOLOGY TRANSFER B: Critical source determination of *Campylobacter* and *Salmonella* in poultry production and processing will allow for the development of intervention strategies thereby permitting the delivery of a safer product to market. Primary customers include industry and researchers.

OBJECTIVE C: Create an efficient means for the extraction of target DNA and design a durable PCR or RT PCR assay for the detection and enumeration of *Campylobacter* spp. in poultry operations and poultry products.

PROGRESS C: A rapid detection system that does not rely upon traditional enrichment and/or selective plating methodology was developed for *Campylobacter*. This PCR-ELISA based technique was found to be capable of identifying and enumerating *Campylobacter* directly from poultry samples in less than one day. Currently, samples being tested for the evaluation and optimization of this rapid technique include, but are not limited to, litter, dander, drag swabs, fecal droppings, water swabs, processing biofilms, and carcass rinses.

IMPACT/TECHNOLOGY TRANSFER C: Rapid detection and enumeration of *Campylobacter* in poultry production and processing will allow for the development of intervention strategies thereby reducing consumer exposure. Primary customers include industry and researchers.

OBJECTIVE D: Assess the role of breeder hens in transmitting *Campylobacter* to corresponding commercial broilers.

PROGRESS D: We document breeder flocks as a source of *Campylobacter* for broilers. *Campylobacter* isolates from breeder and progeny broiler flocks were characterized and compared by short variable region (SVR) *flaA* DNA sequencing. The results of the sequencing provided strong evidence that isolates of *Campylobacter* from both flocks were of clonal origin. In a second study isolates of *Campylobacter jejuni* from a parent flock were found to be of the same clonal origin as *Campylobacter jejuni* isolated from processed carcass rinse. This report provides the first cultural and genetic evidence that *Campylobacter* can pass from one generation to the next in broilers.

IMPACT/TECHNOLOGY TRANSFER D: Epidemiologic tracking of *Campylobacter* from breeder flocks to broiler hens will allow the development of more complete knowledge of the principal source(s) of this organism and will allow for the development of intervention strategies thereby permitting the delivery of a safer product to market. Primary customers include industry and researchers.

OBJECTIVE E: Determine ability of acidifying litter treatments to reduce *Campylobacter* populations associated with broiler chickens during production.

PROGRESS E: Application of acidifying litter treatments could affect pathogen populations in the litter. Two commercially available products, aluminum sulfate (alum) and sodium bisulfate, were tested to determine their effect on *Campylobacter* populations associated with broilers raised on treated litter. The incidence of *Campylobacter* cecal colonization was significantly reduced in all trials by the high level alum treatment. Similarly, no *Campylobacter* was recovered from whole carcass rinse samples associated with these treated pens although control pens were 93, 72 and 32% positive at weeks 1, 4 and 6, respectively. The lower level alum treatment reduced cecal colonization frequency in 2 of the 3 trials while either level of sodium bisulfate treatment significantly reduced colonization in only 1 of the 3 replicate trials.

IMPACT/TECH TRANSFER E: Treatment of litter in poultry production may serve as an effective intervention method to control *Campylobacter*, thus reducing the public exposure. Commercial poultry companies are interested in testing these methods and larger scale field trials are currently being planned and conducted.

OBJECTIVE F: Develop a selective differential agar for isolation and enumeration of *Campylobacter* spp.

PROGRESS F: A more selective and differential agar was developed to facilitate *Campylobacter* enumeration by combining selective antibiotics and triphenyl-tetrazolium chloride (TTC). The new agar (Campy-Line agar, CLA) does not contain blood and is translucent. The contrast of deep red colonies on a translucent background greatly facilitates *Campylobacter* isolation and makes enumeration on light boxes or by electronic means possible.

IMPACT/TECH TRANSFER F: The new agar has been proposed to FSIS for use in enumerating campylobacters associated with broiler carcass rinses. The new agar is being produced by a commercial company for testing purposes. FSIS will evaluate the agar and enumeration method in comparison to their existing procedures. A provisional patent application has been filed by ARS on the agar.

PUBLICATIONS:

Zhu, J., R.J. Meinersmann, K.L. Hiett, and D.L. Evans, 1999. Apoptotic effect of outer-membrane proteins from *Campylobacter jejuni* on chicken lymphocytes. Curr. Microbiol. 38: 244-249.

PROCEEDINGS/ABSTRACTS:

Abeyta, C. and N. J. Stern. 1999. Ribosomal DNA analysis links a *Campylobacter jejuni* illness to a Washington state shellfish growing area reported in 1993. 10th International Workshop on *Campylobacter*, *Helicobacter*, and related organisms. Baltimore, MD. USA.

Bruce, J.L., S.J. Fritschel, N.J. Stern, and S.Brooks. 1999. Interlaboratory comparison of automated ribotyping of *Campylobacter* using the RiboPrinter(registered) system. 10th International Workshop on *Campylobacter*, *Helicobacter*, and related organisms. Baltimore, MD. USA.

Cox, N.A., N.J. Stern, K.L. Hiett, and M.E. Berrang, 1999. Transmission of *Campylobacter jejuni* from breeders to commercial broiler chickens. 10th International Workshop on *Campylobacter*, *Helicobacter*, and related organisms. Baltimore, MD. USA.

Cox, N.A., N.J. Stern, K.L. Hiett and M.E. Berrang. *Campylobacter* passage from hen to offspring through the egg. 1999. Proc. 34th national Meeting on Poultry Health and Processing. Ocean city, MD Oct. 20-22.

Cox, N.A., Stern, N.J., and J.S. Bailey, J. S. Sources of *Salmonella* and *Campylobacter* in poultry and methods to intervene. 1999. 52nd Annual Reciprocal Meat Conference pp 5-7.

Cox, N.A., N.J. Stern, K.L. Hiett, and M.E. Berrang, 1999. Egg transmission of *Campylobacter jejuni* from breeder hens to commercial broilers. General Meeting of the United States Animal Health Association. San Francisco, CA. USA.

Cox, N.A., N.J. Stern, K.L. Hiett, and M.E. Berrang, 1999. Sources of *Salmonella* and *Campylobacter* in Poultry. National Environmental Health Association, Nashville, TN. USA.

Fazil, A., R. Lowman, N. Stern, & A. Lammerding. 1999. A quantitative risk assessment model for *C. jejuni* in chicken. 10th International Workshop on *Campylobacter*, *Helicobacter*, and related organisms. Baltimore, MD. USA.

Line, J. E. 1999. Development of a Selective Differential Agar for Isolation and Enumeration of *Campylobacter* spp. 10th International Workshop on Campylobacters, *Helicobacters* and Related Organisms. Baltimore, MD.

Line, J. E. 1999. Reduced *Campylobacter* Populations Associated with Chickens Raised on Acidified Litter. 10th International Workshop on Campylobacters, *Helicobacters* and Related Organisms. Baltimore, MD.

Meinersmann, R.J., N.J. Stern, J.S. Bailey, P.F. Cray, and K.L. Hiett. 1999. Flagellin short variable region (SVR) sequence for *Campylobacter* typing. 10th International Workshop on *Campylobacter*, Helicobacter, and Related Organisms. Baltimore, MD USA

Stern, N. J., J. S. Bailey, N. A. Cox, S. E. Craven & P. F. Cray. 1999. Flow of *Campylobacter* spp. through U.S. poultry operations. 10th International Workshop on *Campylobacter*, *Helicobacter*, and related organisms. Baltimore, MD. USA.

PATENTS:

Invention report No. 0146.99 "Direct Detection and Enumeration of Pathogenic Bacteria in Livestock Production by Polymerase Chain Reaction (PCR)".

Line, J. E., J. Garrish, and K. Glassmoyer. Patent application filed June 3, 1999. Selective media for isolation and enumeration of Campylobacters.

Line, J. E., N. J. Stern, N. A. Cox, J. S. Bailey, C. Ricks, P. Phelps, and M. Knight. March 3, 1998. In-ovo antibiotic and microbial treatment to diminish Salmonellae populations in avians (#5,722,342).

CAMPYLOBACTER AND EMERGING BACTERIAL FOODBORNE PATHOGENS IN LIVESTOCK: DETECTION AND CONTROL

ARS Contact Persons:
R.B. Rimler
I.V. Wesley

CRIS NUMBER: 3625-32420-004
FSIS CODE: N1MA03
CRIS TERM. DATE: April 2004

National Animal Disease Center
Avian and Swine Respiratory Diseases Unit
Ames, IA
Phone: 515-663-7291
Fax: 515-663-7458
E-mail: iwesley@nadc.ars.usda.gov.

OBJECTIVE A: To improve rapid and sensitive PCR-based techniques to detect and to enumerate *Campylobacter* and other emerging food-borne bacterial pathogens (e.g., *Yersinia, Listeria, Arcobacter*) in livestock and *Campylobacter* and *Salmonella* in turkeys.

PROGRESS A: We developed a PCR-based assays which are specific for the following recognized human pathogens: *Campylobacter jejuni, C. coli, Listeria monocytogenes, Yersinia enterocolitica,* and *Arcobacter butzleri.* Current research efforts have focused on adapting these assays to second-generation fluorogenic 5' nuclease PCR (TaqMan) protocols. We developed a TaqMan assay for *Y. enterocolitica,* which targets the *ail* virulence gene. Future research goals include the application of this assay to quantitate *Y. enterocolitica* in hog feces, tissues, and pork products.

IMPACT/TECHNOLOGY TRANSFER A: These methods will permit the rapid detection and quantitation of human bacterial foodborne pathogens, including *Campylobacter, Yersinia,* and *Listeria monocytogenes* in livestock and *Salmonella* on turkey carcasses. This is critical in evaluating the effectiveness of in-plant HACCP strategies.

OBJECTIVE B: To utilize these PCR-based assays to evaluate on-farm management practices to reduce these human foodborne bacterial pathogens (listed in Objective A) in livestock, especially pigs, and to reduce *Campylobacter* and *Salmonella* in turkeys.

PROGRESS B: We have completed a two-state pilot study to determine the level of carcass contamination of hogs (n=500) raised under all in-all out *versus* continuous flow management practices. This study was funded by FSIS and conducted in collaboration with Iowa State University and North Carolina State University. PCR assays were used for the rapid identification of *C. jejuni, C. coli* and *Y. enterocolitca* during on-farm production and on hog carcasses. The results indicated that carcass contamination was lower in hogs originating from all in-all out *versus* continuous flow

operations. Although *Y. enterocolitica* was infrequently found in tonsils of hogs in Iowa (0%) and North Carolina (2.1%), it was detected in tonsils of hogs from Pennsylvania (30%).

Feed withdrawal is practiced in hogs to reduce the gut contents prior to slaughter. We developed a cannulated pig model to monitor the population of foodborne pathogens in the pig caecum. Colonization of the pig caeca followed direct instillation with *Salmonella* and to a lesser extent *Yersinia.* However, no predictable shift in pathogen levels was seen during interrupted feeding. In contrast, *C. jejuni* infections could not be established in market weight hogs by direct instillation into the caecum.

The final stage of hog production involves the transport of animals to slaughter. Although the majority of animals move directly from the farm to the abattoir, some hogs pass through a hog buying station (lairage) and then to slaughter. In conjunction with colleagues at Iowa State University (McKean) and NADC (Hurd), we are evaluating the role of lairage on the prevalence of *Campylobacter*, *Yersinia*, *A. butzleri,* and *L. monocytogenes* on hog carcasses (n=300).

In collaboration with a major turkey processor in the Midwest we are applying the multiplex PCR assays developed in our lab to monitor the effectiveness of in-plant intervention strategies to reduces carcass pathogen levels. In this study, *C. jejuni*, *C. coli*, *Listeria monocytogenes,* and *A. butzleri* are being monitored in turkey carcasses subjected to various sanitizing protocols.

IMPACT/TECHNOLOGY TRANSFER B: Studies demonstrate the application of rapid microbial detection for evaluating the efficacy of on-farm pathogen reduction strategies. A report detailing the lack of effect of feed withdrawal on caecal populations of *Salmonella* and *Y. enterocolitica* has been given to the National Pork Producers Council. It is anticipated that the TaqMan assay will be used in a portion of USDA-APHIS-NAHMS-Swine 2000 to map the national prevalence of and to identify on-farm risk factors for *Y. enterocolitica* in hogs.

PUBLICATIONS:

Manke, T.R., I.V. Wesley, J.S. Dickson and K.M, Harmon. 1998. Prevalence and genetic variability of *Arcobacter* and *Arcobacter butzleri* in mechanically separated turkey. J. Food Protect. 61:1623-1628.

de Oliveira, S.J., I.V. Wesley, A.L. Baetz, K.M. Harmon, I.T. Ivonyr and M. de Uzeda. 1999 *Arcobacter cryaerophilus* and *Arcobacter butzleri* isolated from preputial fluid of boars and fattening pigs in Brazil. J. Vet. Diagn. Invest. 11(5): (in press)

Oyarzabal, O.A., I.V. Wesley, J.M. Barbaree, L.H. Lauerman and D.E. Conner. 1999. Identification and characterization of *Arcobacter* and *Campylobacter* from retail poultry by physiological testing and the polymerase chain reaction technique. Poultry Sci. 78: (in press)

Rice, W.E., M.R. Rodgers, C.H. Johnson, I.V. Wesley and A.S. Tanner. 1999. Isolation of *Arcobacter* spp. from ground water. Lett. Appl. Microbiol. 28:31-35.

Wesley, I.V. 1999. *Arcobacter/Helicobacter. In:* R. Labbe and J. Santos (eds.), A Guide to Foodborne Diseases. John Wiley and Sons, New York, NY. (in press)

Wesley, I.V., S.K. Franklin, L. Schroeder-Tucker and E.A. Murano. 1999. *Arcobacter. In*: Am. Public Health Assoc. (eds.), Compendium of Methods for the Microbiological Examination of Foods, 4th ed. Edwards Brothers, Ann Arbor, MI. (in press)

Zakharova, N., I.V. Wesley, B. Pasteur, D.E. Berg and K.V. Severinov. 1999. Fused and overlapping rpoB and rpoC genes in *Helicobacter*, *Campylobacter* and related bacteria. J. Bacteriol. 181:3857-3859.

PROCEEDINGS/ABSTRACTS:

Ramos, A.M., J. Dickson, K.M. Harmon and I.V. Wesley. 1998. Rapid detection of *Listeria monocytogenes* in mechanically separated turkey meat. Proc. Annu. Meet. Food Safety Consort., pp. 136-140.

Wesley, I.V. 1998. Overview: Public health significance of *Campylobacter* in livestock and poultry. Proc. Annu. Meet. U.S. Anim. Health Assoc. Vol. 102, pp. 216-234.

Wesley, I.V., J.D. McKean, P. Turkson, P. Davies, S.C. Johnson, T.A. Proescholdt and G.W. Beran. 1998. *Campylobacter* spp. and *Yersinia enterocolitica* in growing pigs in Iowa and North Carolina: A pilot study. Annu. Meet. Food Safety Consort., pp. 141-150.

Wesley, I.V., A.L. Baetz and J. Laufer. 1999. The cannulated pig: A model for monitoring the dynamics of foodborne pathogens *in vivo*. 99th Gen. Meet. Am. Soc. Microbiol., Abstr. #P-51, p. 521.

Wesley, I.V., K.M. Harmon, E. Bush and S.J. Wells. 1999. Distribution of *Campylobacter* and *Arcobacter* in livestock. 10th Int. *Campylobacter*, *Helicobacter* and Related Organisms Workshop, Baltimore, MD, Abstr. #RF-1.

Wesley, I.V., J.D. McKean, P. Turkson, P. Davies, S.C. Johnson, T.A. Proescholdt and G.W. Beran. 1998. *Campylobacter* spp. and *Yersinia enterocolitica* in growing pigs in Iowa and North Carolina: A pilot study. 33rd US-Jpn. Mycoplasm. Panel Meet., p. 22.

Wesley, I.V. 1998. Application of biotechnology to food safety. USDA-Universidad de Nuevo Leon. Monterrey, Mexico, November 9-13, 1998.

DISEASE RELATED PROBLEMS OF POULTRY PRODUCTION AND PROCESSING

ARS Contact Persons:
W.E. Huff, G.R. Huff

CRIS NUMBER: 6226-32000-005
FSIS NUMBER: I-92-2
CRIS TERM.DATE: June 2001

Poultry Production and Product Safety Research Unit
Fayetteville, AR
Phone: 501-575-2104/7966
Fax: 501-575-4202
E-mail: huff@comp.uark.edu

OBJECTIVE A: To determine the etiology of turkey green-liver/osteomyelitis complex (TOC), to evaluate involvement of the immune system in TOC, and to develop methods to reduce the impact of TOC on turkey production.

PROGRESS A: USDA-ARS research on TOC is based on the hypothesis that this disease is a result of host susceptibility to opportunistic bacterial infection caused by a high response to the stressors of modern turkey production in a sub-population of male turkeys. This is supported by the fact that TOC is only a problem in male turkeys, rarely in females, and the endocrine alterations involved in stress are influenced by gender. We have established an experimental model for TOC which reproduces all FSIS recognized lesions. This model uses treatment with dexamethasone (DEX), followed by respiratory infection with *Escherichia coli.* Multiple sequential treatments with DEX can also reproduce TOC and in this case, *Staphylococcus aureus* is the primary bacterial isolate. We have shown that supplementation with vitamin D_3 can protect turkeys from disease in this model, but that vitamin C and β-glucan had no effect We have also determined that handling of turkey poults for the first 10 days after hatch can induce changes in hematology and immune function which protect the birds from disease later in life.

IMPACT/TECH TRANSFER A: Our model has enabled the evaluation of nutritional and management-related solutions to this problem. This model is the first to demonstrate a respiratory origin for the bacteria that cause TOC. We have established that TOC can result from stress-related immune dysfunction and therefore might be prevented by decreasing stress, by nutritional immunomodulation, or by the genetic manipulation of the stress response. Since *E. coli* air sacculitis/septicemia is one of the most important turkey diseases, treatments resulting from this model will have wide impact on turkey health. The ability of vitamin D_3 to protect birds from stress-induced immunosuppression may provide a low-cost solution to TOC as well as an alternative to antibiotic usage. This model could also be employed in the study of the effects of stress on other poultry diseases including Salmonellosis, Campylobacteriosis, cellulitis, and ascites. leading to the reduction of pathogen contamination of animal food products. Immunomodulators and stress-reduction procedures identified using this model, and the potential of genetically selecting birds with

a low response to stress, should improve resistance to bacterial infection resulting in less need for antibiotic usage in animal production.

OBJECTIVE B: To isolate and characterize the etiological agent of proventriculitis in broilers.

PROGRESS B: Proventriculitis is a problem of food safety significance because rupture of the proventriculus during processing causes carcass contamination with intestinal contents. We have established that homogenates of affected proventricular material will cause proventriculitis when fed to day-old broilers and we have isolated a bacterial and a suspect viral agent which together can interact to reproduce this disease Progress this year has been made toward characterizing the bacterial isolate. These studies suggests that proventriculitis is not caused by a single agent but results from a variety of insults to the proventriculus.

IMPACT/TECH TRANSFER B: We are co-investigators on a patent application for development of a vaccine to prevent proventriculitis using IBDV isolated from proventriculus homogenates. Current work is targeted at further characterizing the agents responsible for proventriculitis.

OBJECTIVE C: The development of methods to increase intestinal strength of poultry.

PROGRESS C: Intestines can become weakened and easy to tear due to the effects of disease, mycotoxins, and diet. Mechanical evisceration can then result in torn intestines which contaminate carcasses and increase the spread of potential pathogens throughout the processing plant. We have developed a sensitive method for accurately measuring intestinal strength not only to document the effects of agents which decrease intestinal strength, but also to evaluate ways to increase intestinal strength. We have evaluated many approaches to increasing intestinal strength such as feeding short chain fatty acids like propionic acid, using the astringents tannic acid and alum, β-glucan, betaine, and trying to buffer the intestinal tract with calcium carbonate. To date, these approaches have proven to be ineffective in increasing intestinal strength. Preliminary data collected this year suggest that some improvement in intestinal strength can occur when birds are fed high fiber diets. Additional studies are ongoing to determine the efficacy of dietary fiber to increase intestinal strength.

IMPACT/TECH TRANSFER C: The methodology developed for measuring intestinal strength is a valuable tool for research into intestinal disease. Development of methods to increase intestinal strength would decrease the cost of poultry processing and increase product safety. We are using this technology to continue to search for a practical way to increase intestinal strength in poultry.

PUBLICATIONS:

Huff, G.R., W. E. Huff, J. M. Balog, and N. C. Rath, 1999. Sex differences in the resistance of turkeys to *Escherichia coli* challenge after immunosuppression with dexamethasone. Poultry Sci. 78:38-44.

Huff, G.R., W.E. Huff, J. M,. Balog, and N.C. Rath, 1999. The effect of a second dexamethasone treatment on turkeys previously challenged in an experimental *Escherichia coli* respiratory model of turkey osteomyelitis complex. Poultry Sci. 78:1116-1125.

PROCEEDINGS/ABSTRACTS:

Huff, G.R., W.E. Huff, J.M. Balog, and N.C. Rath, 1999. The effects of dexamethasone in an experimental model of turkey osteomyelitis complex. p. 63-67 in: Proceedings 48th Western Poultry Disease Conference, Vancouver, Canada.

Huff, W. E., G. R. Huff, J. M. Balog, and N. C. Rath, 1999. Studies on intestinal strength of poultry. p. 22-23 in: Proceedings of the 48th Western Poultry Disease Conference. Vancouver, British Columbia, Canada.

Huff, G.R., W.E. Huff, N.C. Rath, and J.M. Balog, 1999. The effects of supplemental vitamin C and vitamin D_3 on stress-related bacterial infections in turkeys. Proceedings of the Arkansas Nutrition Conference.

Huff, G.R., W.E. Huff, J.M. Balog, and N.C. Rath, 1998b. The effect of vitamin D_3 on the incidence of turkey osteomyelitis complex in an experimental *Escherichia coli* respiratory infection. Poultry Sci. 77(Suppl. 1)73.

Huff, G.R., W.E. Huff, N.C. Rath, and J.M. Balog, 1999. The effects of dexamethasone immunosuppression on bactericidal activity of turkey monocytes. 99th General Meeting of the American Society for Microbiology. Chicago, IL.

DEVELOPMENT AND EVALUATION OF PROBIOTICS FOR CONTROL OF BOVINE AND PORCINE ENTERIC PATHOGENS

ARS Contact Persons: **L.H. Stanker, D.J. Nisbet, R.B. Harvey, R.C. Anderson**

Food and Feed Safety Research Unit
Food Animal Protection Research Laboratory
College Station, TX
Phone: 409-260-9484
Fax: 409-260-9332
E-mail: nisbet@ffsru.tamu.edu

CRIS NUMBER: 6202-42000-010
FSIS CODE: C1AM04
CRIS TERM DATE: October 2001

OBJECTIVE A: Develop a competitive exclusion culture to prevent and/or control enteric pathogens in swine, and identify critical control points where competitive exclusion or other appropriate intervention strategies can be incorporated to control enteropathogens in commercial production of swine.

PROGRESS A: A defined competitive exclusion culture has been developed for swine. The individual isolates from this culture have been isolated and a recombined culture has been developed that has the same efficacy as the original culture. Studies have been performed that show this culture significantly reduces shedding of *Salmonella* in baby and weaned pigs. Additionally this culture has been shown to significantly reduce mortality associated with pathogenic *E. coli* in very early weaned pigs.

IMPACT/TECH TRANSFER A: The patent for the original swine competitive exclusion culture should issue in 1999. Additionally a patent for the use of this culture in very early weaned pigs in combination with liquid milk replacer has been filed. We will initiate patent protection during the next year on the process developed for recombining the individual bacterial isolates. An INAD (Investigational New Animal Drug) permit has been received from the Food and Drug Administration for commercial field trials. These trials should begin within FY 2000. Once the swine patent has issued our CRADA partners will begin negotiations for exclusive licensing.

OBJECTIVE B: Perform epidemiological studies on the presence of *Salmonella*, *Campylobacter*, and *Arcobacter* in commercial swine.

PROGRESS B: Epidemiological studies are being performed in cooperation with a local integrated swine producer in Texas. Studies identifying the Incidence of *Salmonella* and *Campylobacter* have been performed in various phases of swine production. New studies on the incidence of *Arcobacter* have recently been initiated. Manuscripts reporting the incidence, serotypes and antimicrobial

resistance profiles have been submitted to journals for publication. Additionally, several presentations have been given at scientific meetings during the past year.

IMPACT/TECH TRANSFER B: The data from the epidemiology studies should give us a more thorough understanding of the critical control points in swine production, as well as the effect of antimicrobial use on the incidence of antibiotic resistant enteropathogens. The epidemiological data is also being used in order to identify swine production facilities where our competitive exclusion culture can be tested in field trials.

OBJECTIVE C: Using continuous-flow cultures of mixed microbial flora obtained from the gastrointestinal tract of food producing animals, investigate interactions between normal flora and enteropathogens as well as the effects of antimicrobials on normal flora population dynamics. Additionally studies have been initiated exploring the effects of vancomycin on vancomycin resistant acquisition in GI tract enterococci.

PROGRESS C: A continuous culture of mixed cecal microflora has been shown to be an excellent model of the cecae in regard to *Salmonella* colonization. A manuscript has been accepted in the peer reviewed literature that documents similar *Salmonella* survivability in the in vitro model as found in the cecae. Currently we are using this model to show the effects of vancomycin on changes in vancomycin resistant enterococci within the continuous flow culture.

IMPACT/TECH TRANSFER: By using GI tract models to better understand interactions between microbial populations information can be obtained to make scientifically sound decisions on the effects of antimicrobial use on antibiotic resistant acquisition. This model should provide other scientists and agencies a risk assessment tool to make decisions on antimicrobial use in food producing animals. In addition, these mixed microbial models should provide information on the effects of management practices such as feed withdrawal on the integrity of the host microflora and its ability to protect against enteropathogen colonization.

OBJECTIVES D: Develop a microbial based intervention strategy to eliminate or decrease colonization levels by pathogenic *E. coli* such as *E. coli* O157:H7 in the rumen of cattle.

PROGRESS D: Studies have shown that chlorate when provided in low concentrations can selectively kill *E. coli* O157:H7 and *Salmonella* DT104 in in vitro models using microflora obtained from the rumen of cattle. This substrate also has no apparent effect on the host normal flora. Live animal studies using cannulated dairy cows have shown that chlorate significantly reduces rumen and fecal concentrations of *E. coli*. The use of chlorate as a novel compound to be used in pre-slaughter cattle to purge the GI tract of enteropathogens is currently in the patent process.

IMPACT/TECH TRANSFER D: The cattle industry is very interested in this intervention strategy and this research has been well received. Currently we are identifying an industry CRADA partner to cooperate in field trials and further develop the product.

PUBLICATIONS:

Anderson, R.C., L.H. Stanker, C.R. Young, S.A. Buckley, K.J. Genovese, R.B. Harvey, J.R. DeLoach, N.K. Keith and D.J. Nisbet. 1999. Effect of competitive exclusion treatment on colonization of early-weaned pigs by *Salmonella* serovar *cholerasuis*. Swine Health Prod. 7 (4):155-160.

Martin, S.A., M.N. Streeter, D.J. Nisbet, G.M. Hill and S.E. Williams. 1999. Effects of DL-malate on ruminal metabolism and performance of cattle fed a high concentrate diet. J. Anim. Sci. 77:1008-1015.

PROCEEDINGS/ABSTRACTS:

Anderson, R.C., R.B. Harvey, K.J. Genovese, L.H. Stanker, J.R. DeLoach and D.J. Nisbet. 1999. Long term shedding of *Salmonella cholerasuis* following experimental infection of very young piglets. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp.16-17.

Anderson, R.C., R.B. Harvey, K.J. Genovese, L.H. Stanker, J.R. DeLoach and D.J. Nisbet. 1999. Comparison of two enrichment schemes for qualitative recovery of *Salmonella* serovar *cholerasuis* from rectal swabs collected from neonatal and early weaned pigs. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp. 13-15.

Anderson, R.C., K.J. Genovese, R.B. Harvey, L.H. Stanker, N.K. Kieth, J.R. DeLoach and D.J. Nisbet. 1999. Effect of competitive exclusion on transmission of *Salmonella cholerasuis* between early weaned pigs. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp. 18-21.

Genovese, K.J., R.C. Anderson, D.J. Nisbet, R.B. Harvey, V.K. Lowry, L.H. Stanker and M.H. Kogut. 1999. Preliminary experiments involving *Salmonella*-immune lymphokine in vivo effects on neutrophil function in weaned pigs and *Salmonella cholerasuis* organ invasion in neonatal pigs. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp.42-45.

Harvey, R.B., R.C. Anderson, L.A. Farrington, R.E. Drolesky, K.J. Genovese, L.H. Stanker and D.J. Nisbet. 1999. Comparison of initial enrichment on the recovery of salmonellae from swine lymph nodes and cecal contents at slaughter. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp. 51-52.

Harvey, R.B., L.A. Farrington, S.A. Buckley, R.E. Droleskey, L.H. Stanker, D.J. Nisbet and P.D. Inskip. 1999. Epidemiological survey of antimicrobial-resistant *Salmonella* in market-age swine. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp. 252-256.

Nisbet, D.J., R.C. Anderson, R.B. Harvey, K.J. Genovese, J.R. DeLoach and L.H. Stanker. 1999. Competitive exclusion of *Salmonella* serovar Typhimurium from the gut of early weaned Pigs. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp.80-82.

Harvey, R.B., L.A. Farrington, R.E. Droleskey, R.C. Anderson, L.H. Stanker and D.J. Nisbet. 1999. Comparison of two commercially available ELISA kits for detection of salmonellae in swine lymph nodes and cecal and fecal contents. Int'l Symposium on epidemiology and control of *Salmonella* in Pork. pp. 53-56.

STRATEGIES TO CONTROL SWINE PARASITES AFFECTING FOOD SAFETY

ARS Contact Persons:
Joseph F. Urban, Jr.,
Joan K. Lunney
Dante S. Zarlenga

CRIS NUMBER: 1265-32000-049
FSIS NUMBER: B2AA04,05
CRIS TERM. DATE: September 2000

Immunology and Disease Resistance Laboratory
Livestock and Poultry Sciences Institute, ARS, USDA
Building 1040 Room 105
Beltsville, Maryland 20705-2350
Phone: 301-504-8765 or -8201
Fax: 301-504-5306
E-mail: jurban@lpsi.barc.usda.gov

OBJECTIVE A: Reduce transmission of foodborne pathogens of swine by defining cytokine-regulated immune mechanisms that protect pigs against parasites that threaten food safety and by developing molecular reagents that accurately identify zoonotic genotypes.

PROGRESS A: Immunological control of *Toxoplasma gondii* infections in swine is a target for preharvest management of this foodborne pathogen. Pigs that survived a deliberate infection of oocysts derived from farm cats were evaluated for the production of interferon-gamma (IFNg). Rapid expression of IFNg was associated with pigs that limited numbers of *T. gondii* in their tissues after challenge infection. Vaccines that induce high levels of IFNg are likely to enhance protections against infection.

A series of primers have been developed to recognize genotypes/species of *Trichinella.* These specific reagents will be especially important in identifying contamination of pig and horse meat with *Trichinella* that is freeze-resistant. Freezing is currently the most useful commercial and household method for reducing the risk of infection.

Primers have been developed to determine the level of several important pig cytokines including IL-2, IL-4, IL-5, IL-10, IL-12, IL-12 receptor, IL-15 and IFNg. These reagents will be used to evaluate the immune status of pigs infected with *T. gondii* and others infected with *Trichuris suis* that predisposes pigs to secondary infection with *Campylobacter jejunii.* This work supports additional studies in mice that predict a stereotypical cytokine response pattern to different parasitic infections that may be appropriate or inappropriate to the level of resistance to infection.

IMPACT/TECH TRANSFER A: The diagnostic test for differentiating *Trichinella* genotypes has been accepted for publication and is available for world-wide application through the International Trichinella Reference Center in Rome, Italy.

Primers for pig cytokines are currently available for collaborative studies with scientists in Beltsville, Michigan State University and Pharmacia/Upjohn. These reagents will provide information on more effective vaccination protocols against *T. gondii* and other zoonotic infections of swine.

PUBLICATIONS:

Appleyard, G.D., D. Zarlenga, E. Pozio, and A.A. Gajadhar. 1999. Differentiation of Trichinella genotypes by PCR using sequence-specific primers. J. Parasitol. 85:556-559.

Gause, W.C., M. Ekkens, D. Nguyen, V. Mitro, F.D. Finkelman, R. Greenwald, and J.F. Urban. 1999. The development of CD4+ T effector cells during the type 2 immune response. Immunologic Res. 20:55-65.

Gause, W.C., Ekkens, M., Nguyen, D., Mitro, V., Finkelman, F.D., Greenwald, R., and Urban, J.F. 1999. The development of CD4+ T effector cells during the type 2 immune response. Immunologic Res. 20:55-65.

Greenwald, R.J., J.F. Urban, M.J. Ekkens, S.J. Chen, D. Nguyen, H. Fang, F.D. Finkelman, A. Sharpe, and W.C. Gause. 1999. B7-2 is required for the progression but not the initiation of the type 2 immune response to a gastrointestinal nematode parasite. J. Immunol. 162:4133-4139.

Mansfield, L.S., D.E. Hill, and J.F. Urban. 1998. Lymphoglandular complexes process antigen in the distal colon in swine. In: Cytokines, cholera, and the gut. (Ed: Keusch, G.T. & Kawakami, M.). IOS Press, Ohmsha pp 185-195.

Mansfield, L.S., W.C. Gause, F.D. Finkelman, and J.F. Urban. 1999. Gastrointestinal nematodes and the immune system. *In:* Effects of Microbes on the Immune System. Eds: Cunningham, M.W. and Fujinamni, R.S. Lippincott/William & Wilkins, Philadelphia, PA.

Solano-Aguilar, G and J.K. Lunney. 1999. Mucosal immune responses in neonatal swine. Immunol. Letters 69:171.

IDENTIFICATION AND MAPPING OF GENES INVOLVED IN PARASITIC DISEASE RESISTANCE/SUSCEPTIBILITY

ARS Contact Person:
Joan K. Lunney

CRIS NUMBER: 1265-31320-012
FSIS NUMBER: B2AA05
CRIS TERM. DATE: September 2000

Immunology and Disease Resistance Laboratory
Livestock and Poultry Sciences Institute, ARS, USDA
Building 1040 Room 105
Beltsville, Maryland 20705-2350
Phone: 301-504-8201
Fax: 301-504-5306
E-mail: jlunney@lpsi.barc.usda.gov

OBJECTIVE: Identify and breed livestock and poultry that are genetically resistant to parasite infections.

PROGRESS: Outbred swine and genetically defined minipigs, pigs with known swine leukocyte antigen (SLA) haplotypes, were challenged with low level infections with oocysts of the foodborne parasite *Toxoplasma gondii* to determine parasite resistant pigs could be identified. Several inbred pigs which were resistant were identified. No association of resistance with SLA haplotype was found. More detailed genotyping studies will be required to determine the genes which endow the pigs with parasite resistance.

Tissue samples from each group of pigs were saved so that immune mechanisms controlling this resistance could be defined using assays for cytokine expression. Tests indicate that production of the immune stimulatory cytokine, interferon-γ, is associated with resistance. Once data has been collected on enough individuals, mapping will be pursued to determine what other genes and immune factors encode such resistance.

IMPACT/TECH TRANSFER: These studies should help breeders to reduce costs of drug and vaccine treatments by selecting for parasite resistant stock. In areas where these parasitic diseases cannot be eliminated, this alternate approach should result in healthier pigs and should help prevent parasite contamination of pork products.

PUBLICATIONS:

Zhao F., S. Ambady, F.A. Ponce de Leon, L.M. Miller, J.K. Lunney, D.R. Grimm, L.B. Schook, and C.F. Louis. 1999. Microsatellite markers from a microdissected swine chromosome 6 genomic library. Anim. Genet. 30:251-255.

PREHARVEST CONTROL OF ZOONOTIC PARASITES IN SWINE

ARS Contact Persons:
H. R. Gamble
J. P. Dubey

CRIS NUMBER: 1265-32000-055
FSIS CODE: B4AI01
CRIS TERM. DATE: September 2003

Livestock and Poultry Sciences Institute
Parasite Biology and Epidemiology Lab
Beltsville, MD
Phone: 301-504-8300
Fax: 301-504-6273
E-mail: rgamble@lpsi.barc.usda.gov

OBJECTIVE A: To develop tools necessary for implementation of a pre-harvest certification program for trichinae infection in pigs.

PROGRESS A: Multiple states participated in a study to identify trichinae-infected herds which could be used to validate farm auditing procedures. A total of 14,686 blood or tissue samples were tested by digestion or ELISA. Of the five states participating, New Jersey, Pennsylvania and Ohio had no positive samples. A total of 3 samples were positive by serology from 3646 samples collected in Indiana and 5 samples were positive by serology from 3617 samples collected in Illinois. No positive farms were confirmed. Due to the absence of trichinae infection in high risk areas, further efforts to validate a farm audit were discontinued. A pilot project to verify the process of certification will be initiated in Fall, 1999. This pilot will incorporate tools developed by ARS and in cooperation with APHIS, FSIS and the National Pork Producers Council. Based on the outcome of the pilot, the certification process for trichinae-free pork production will be launched as a national program in 2000.

IMPACT/TECH TRANSFER: The results of ARS studies support certification as a method for assuring the safety of pork with respect to trichinae. Certification for trichinae will serve as a model for programs for other zoonotic diseases.

OBJECTIVE B: Provide training and quality control in a program for the inspection of pork and horsemeat for export to the European Union and Russia; to provide program support in the form of evaluating and improving inspection methods for trichinae in pork and horsemeat.

PROGRESS B: The ARS administers a training and quality control program which includes 9 pork packers, 4 horse packers and 3 feral swine packers which ship certified trichinae-free products to Europe and Russia. Four training sessions are held each year for personnel at participating plants to become certified. On a quarterly basis, check samples are prepared and distributed to all certified trichinae analysts. Accurate analysis of check samples allows continued certification of these inspectors. Research projects to determine the effectiveness of digestion and serology methods for the detection of trichinellosis, primarily in pigs, continue to be performed. This research is used in

making modifications to the existing program and is conducted in cooperations with scientists in Canada, Europe and Russia. In FY99, results of experiments were published which describe optimal conditions for performance of pooled sample digestion methods. These studies were initiated due to discrepancies which exist in established methods used in the EU and Russia.

IMPACT/TECH TRANSFER: The results of this program and related research is the assurance of market access for fresh pork products in Europe and Russia.

OBJECTIVE C: Determine the prevalence of *Toxoplasma gondii* in horses slaughtered for food in North America.

PROGRESS C: Serum samples from 1788 horses slaughtered for food in North America were tested for antibodies to *Toxoplasma gondii* using the modified direct agglutination test (MAT). Antibodies to *T. gondii* were found by the MAT in 124 (6.9%) of 1788 sera; the titers were 1:20 (69 horses), 1:40 (37 horses), 1:80 (9 horses), and ≥1:160 (9 horses). A total of 339 selected horses were also tested by the Sabin-Feldman dye test (DT). Dye test antibodies were found in 54 horses with titers of 1:10 (29 horses), 1:20 (12 horses), 1:40 (4 horses) and 1:80 (9 horses). There was no correlation between the DT and the MAT.

IMPACT/TECH TRANSFER: Overall, the prevalence of *T. gondii* in horses was low and thus the risk of acquiring toxoplasmosis from eating horse meat may not be great. This information will be useful to FSIS with respect to meat inspection and testing of horse meat for export to France.

OBJECTIVE D: Determine the prevalence of *Toxoplasma gondii* in pigs raised in high risk management systems.

PROGRESS D: To determine regional prevalence of infection with *Toxoplasma gondii*, 1897 pigs from 85 farms in 5 New England states were tested using a modified direct agglutination test. Sera were diluted 1:25 and a titer at this dilution was indicative of *T. gondii* infection. Farm management questionnaires were completed at the time of blood collection and were used to develop descriptive statistics on farms tested and to determine measures of association for risk factors for the presence of *T. gondii*-seropositive pigs. A total of 900 seropositive pigs were identified for a prevalence rate of 47.4%. Of 85 herds tested, 77 had at least 1 positive pig for a herd prevalence rate of 90.6%. Within herd prevalence ranged from 4-100% (mean = 48.4%). All farms studied had one or more risk factors for exposure to *T. gondii*. However, statistical associations with individual risks could not be made, most likely due to the extremely high prevalence. Three other states contributed samples to test for *Toxoplasma* infection (New Jersey, Indiana and Illinois). Samples were obtained from slaughter sows raised primarily outdoors. Prevalence rates were 33.1% (498/1504) for New Jersey, 43.2% (1574/3646) for Indiana, and 20.7% (723/3498) for Illinois.

IMPACT/TECH TRANSFER: The results obtained here suggest that education on farm management practices to reduce exposure to *T. gondii* should be targeted to include small producers and those farms that raise pigs outdoors.

OBJECTIVE E: Risk factors for infections with *Toxoplasma gondii* for residents and workers on swine farms in Illinois.

PROGRESS E: Risk factors for *Toxoplasma gondii* infection in workers and residents of swine farms were studied on 43 farms in Illinois. Blood samples were collected from 174 adults in 1993. The *T. gondii* seroprevalence was 31%. An interview was conducted with each participant, obtaining information on demographic characteristics and behaviors suspected to affect the risk of *T. gondii* infection. Factors associated with increased risk of *T. gondii* seropositivity were a higher number of seropositive cats trapped on the farm, male sex, rearing pigs on pasture and gardening. Factors associated with a decreased risk were handling of pig feed and presence of cats inside the pig facilities. Thus, infection of cats with *T. gondii* increased the risk of human infection, and contact with soil was a likely mechanism for transmission. The increased risk of seropositivity in males is attributed to less attention paid to cleanliness in food preparation and eating.

IMPACT/TECH TRANSFER: This information will be useful to FSIS and public health workers regarding epidemiology of toxoplasmosis in rural America.

OBJECTIVE F: Validate the ELISA test for toxoplasmosis in swine.

PROGRESS F: Heart tissue, diaphragm tissue and serum are being collected from a slaughter facility in Pennsylvania for evaluation in a series of detection methods for *Toxoplasma* infection in pork. To confirm infection, tissue is fed to cats and shedding of oocysts is monitored. Serum is evaluated by a modified agglutination test which is a standard method for *Toxoplasma* detection. Samples are also tested using a whole tachyzoite ELISA, in cooperation with Safe-Path Laboratories (Minneapolis, MN). In preliminary studies the ELISA compares favorably with results of bioassay and outperforms the MAT. Further studies will be conducted. Several recombinant antigens have been evaluated for use in an ELISA for detecting *Toxoplasma* infection in pigs. Of the antigens tested, the P30 tachyzoite surface antigen appears to work best.

IMPACT/TECH TRANSFER: These studies will result in the availability of a highly reliable and rapid method of testing pigs for infection with *Toxoplasma*.

PUBLICATIONS:

Dubey, J.P., P.Thulliez, S. Romand, O.C.H. Kwok, S.K. Shen, and H.R. Gamble. 1999. Serologic prevalence of *Toxoplasma gondii* in horses slaughtered for food in North America. Vet. Parasitol. (in press)

Gamble, H.R. and E. Bush. 1998. Seroprevalence of *Trichinella* infection in domestic swine based on the National Animal Health Monitoring System's 1990 and 1995 Swine surveys. Vet. Parasitol. 80: 303-310.

Gamble, H.R. 1998. Trichinellosis. In: OIE Manual of Standards for Diagnostic Tests and Vaccines. Chapter 3.5.3, pp. 477-480.

Gamble, H.R. 1999. *Trichinella. In:* Encyclopedia of Food Microbiology. Academic Press, London.

Gamble, H.R., R.C. Brady, L.L. Bulaga, C.L. Berthoud, W.G. Smith, L.A. Detweiler, L.E Miller, and E.A. Lautner. 1999. Prevalence and risk factors for trichinellosis in domestic pigs in the northeastern United States. Vet. Parasitol. 82: 59-69.

Gamble, H.R., R.C. Brady, and J.P. Dubey. 1999. Prevalence of *Toxoplasma gondii* infection in domestic pigs in the New England states. Vet. Parasitol. 82: 129-136.

Gamble, H.R. 1999. Factors affecting the efficiency of pooled sample digestion for the recovery of *Trichinella spiralis* from muscle larvae. Int. J. Food Microbiol. 48: 73-78.

Gamble, H.R. 1999. Trichinae.. In: Pork Facts: Quality and Safety, American Meat Science Association.

Pozio, E., C.M.O. Kapel, and H.R. Gamble. 1999. Specificity and sensitivity of random amplified polymorphic DNA analysis for the identification of single larvae of *Trichinella.* Parasitol. Res. 85: 504-506.

Weigel, R.M., J.P. Dubey, and D. Dyer. 1999. Risk factors for infections with *Toxoplasma gondii* for residents and workers on swine farms in Illinois. Am. J. Trop. Med. Hyg. 60:793-798.

CONTROL AND PREVENTION OF CRYPTOSPORIDIOSIS

ARS Contact Persons:
Ronald L. Horst
James A. Harp

CRIS NUMBER: 3625-32000-040
FSIS CODE: N2AM01
CRIS TERM. DATE: February 2004

National Animal Disease Center
Metabolic Diseases and Immunology Research Unit
Ames, IA 50010
Phone: 515-663-7533
Fax: 515-663-7669
E-mail: jharp@nadc.ars.usda.gov

OBJECTIVE A: Develop methods to prevent or minimize *Cryptosporidium parvum* infection in cattle.

PROGRESS A: Intense, early exposure of calves to *C. parvum* in the highly contaminated environment present on most production facilities has been shown to overwhelm any potential protection induced by the first generation vaccine produced in this lab. We are now working to develop a second generation vaccine that will provide quicker protection and thus be more effective in a production setting. We have obtained genes coding for *C. parvum* proteins and immune-stimulating molecules. We are testing DNA constructs of these genes for ability to evoke protective immune responses in mice and calves. We will insert promising constructs into bacterial vectors that will be tested as oral vaccines in newborn animals for their ability to induce a rapid immune response to *C. parvum.* In additionally, we are screening candidate drugs that interfere with a unique pathway in *C. parvum* metabolism. These drugs may prove to be useful in treating or preventing *C. parvum* infection in calves.

IMPACT/TECH TRANSFER A: Our published findings indicate that protection of calves from on-farm contamination with *C. parvum* will require better vaccines and treatments along with improved hygiene and management of calves by producers to reduce early exposure to the parasite. These efforts will reduce the levels of preharvest contamination, and reduce the chance of *C. parvum*-contaminated water and food reaching the consumer. Development of second generation vaccines and efficacious drug treatment will lead to the control of *C. parvum* infection on the farm, and result in a reduced risk of contaminated food and water for the American consumer.

PUBLICATIONS:

Atwill, E.R., J.A. Harp, T. Jones, P.W. Jardon, S. Checel and M. Zylstra. 1998. Evaluation of periparturient dairy cows and contact surfaces as a reservoir of *Cryptosporidium parvum* for calfhood infection. Am. J. Vet. Res. 59:1116-1121.

Harp, J.A. and J.P. Goff. 1998. Strategies for the control of *Cryptosporidium parvum* infection in calves. J. Dairy Sci. 81:289-294.

PROCEEDINGS/ABSTRACTS:

Harp, J.A., D. Akili and B.A. Pesch. 1999. Changes in murine intestinal epithelium following *Cryptosporidium parvum* infection. 6th International Workshops on Opportunistic Protists, Raleigh, N.C.

Waters, W.R., J.A. Harp, M.J. Wannemuehler, F.A. Ahrens, B. Frydman and N. Yarlett. 1998. Efficacy of a spermine analogue in the treatment and prevention of *Cryptosporidium parvum* infection of TCR-α-deficient mice. Conf. Res. Workers Anim. Dis. 79:108.

Waters, W.R., J.A. Harp, M.J. Wannemuehler, N.Y. Carbajal, and I.A. Casas. 1999. Effects of *Lactobacillus reuteri* on *Cryptosporidium parvum* infection of gnotobiotic TCR-α-deficient mice. 6th International Workshops on Opportunistic Protists, Raleigh, N.C.

Waters, R., J. Harp, M. Wannemuehler, N. Carbajal and I. Casas. 1999. *Lactobacillus reuteri* and intestinal integrity. XIII International Symposium of Gnotobiology, Stockholm, Sweden.

PREVENTION & THERAPY FOR PROTOZOAN PARASITES AFFECTING FOOD ANIMALS, FOOD SAFETY, PUBLIC HEALTH

ARS Contact Persons:	CRIS NUMBER:	1265-32000-050
R. Fayer, M.C. Jenkins,	FSIS CODE;	B2M01-03
J.F. Urban, Jr., J. Higgins	CRIS TERM. DATE:	October 2000

Immunology and Disease Resistance Laboratory
Livestock and Poultry Sciences Institute, ARS, USDA
Building 1040 Room 105
Beltsville, Maryland 20705-2350
Phone: 301-504-8201/6443
Fax: 301-504-5306
E-mail: jhiggins@lpsi.barc.usda.gov

OBJECTIVE A: Prevent illness in food animals, control food contamination and protect public health from enteric protozoan parasites *Cryptosporidium parvum* and *Cyclospora cayetanensis*.

PROGRESS A: Research to characterize the extent and distribution of *Cryptosporidium parvum* oocysts in Chesapeake Bay oysters indicates that oysters from all bars being monitored (n = 6 total) are contaminated. Oocysts recovered from oysters are viable, according to results obtained from neonatal mouse infection assays. The use of a nested PCR protocol, based on a parasite gene identified by ARS researchers, improves the detection of oocysts in oysters. Sequencing of the PCR products from some oysters indicates that oocysts are *C. parvum*, bovine genotype. A collaborative agreement with personnel at the Naval Medical Research Unit-2 in Jakarta, Indonesia, provides ARS researchers with oocysts of *Cyclospora cayetanensis*. Oocysts have been characterized using gene sequencing techniques, and cross-reactive antibodies raised against other species of enteric protozoa. Experiments are ongoing to propagate sporulated oocysts in a variety of laboratory animals, and to construct genomic and cDNA libraries for molecular biology-based analyses.

A "real-time", fluorescent-probe based PCR method was developed for the detection of *C. parvum* oocysts in water. When viable oocysts [filtered from larger amounts of water] are used as template, the assay provides results in under two hours, with a detection limit of 5 oocysts per reaction for tap water. For more turbid water (e.g., creek water) the detection limit is 100 oocysts per reaction.

A reverse-transcriptase (RT) PCR assay was developed to detect viable *Cryptosporidium* oocysts in a variety of sample preparations. Only viable oocysts generate a positive reaction, and when oocysts maintained at 4°C for 6, 3, and 1 months are assayed, there are distinct differences in signal intensity, indicating that the assay can be used to determine the age of oocysts. The assay also exhibited a correlation between the intensity of the RT-PCR signal, and the ability of the oocysts to infect mice. The RT-PCR assay may therefore supplant the expensive and laborious mouse infection assay, as a means of determining oocyst infectivity and virulence.

Immunological reagents, based on several *Cryptosporidium* proteins identified by ARS researchers, are being developed for use in detecting oocysts in a variety of sample types. One protein (Cp41) is a constituent of the oocyst wall that appears to be species-specific; using genetic engineering techniques, a recombinant form of this protein is being investigated for use in diagnostic assays. In addition, the use of cell culture and immunofluorescent microscopy to determine the viability of C. parvum oocysts, are being evaluated.

Recently, outbreaks of cryptosporidiosis have occurred in recreational water parks despite the use of acceptable levels of chlorination and filtration. IDRL and CDC scientists conducted experiments to determine the efficacy of conventional chlorination to inactivate *C. parvum* oocysts. The results indicate that even minute amounts of fecal material in the water could inhibit the ability of chlorine treatments to render oocysts non-infectious.

ARS scientists evaluated the utility of irradiation to inactivate *C. parvum* oocysts. A dose-response curve was generated, and used to determine the gamma-irradiation level necessary to render oocysts non-infectious.

IMPACT/TECH TRANSFER A: By detecting *Cryptosporidium* in oysters and mussels we have shown that shellfish can be good indicators of fecal pollution of surface waters and that they pose a public health risk if eaten raw. Utilizing PCR testing for detection of *Cryptosporidium* increases our ability to detect lighter infections and to differentiate species infectious for humans versus those noninfectious for humans and in some cases to determine the source of the fecal contamination. Information generated by these studies is used by personnel at the Maryland Department of Natural Resources in their decisions to open or close select oyster bars.

The RT-PCR assay for oocyst viability, and the generation of immunological reagents for C. parvum detection, will greatly improve the ability of water resource personnel to detect this pathogen. A CRADA with the American Water Works Research Foundation will be used to transfer these technologies to end-users in the water industry. The development of a "real time" PCR assay for detection of *Cryptosporidium* in drinking water also promises to improve the ease and rapidity of diagnostic assays used by water treatment installations. A CRADA with the Water Environment Research Foundation will fund the fabrication of a portable thermal cycler instrument, and allow us to evaluate the assay for use in "on-site" applications.

A number of *C. parvum* proteins have been identified as potential targets for immunological-based diagnostic assays. Patents have been obtained (Cp 15/16), or are pending, for these proteins.

PUBLICATIONS:

Carpenter, C., R. Fayer, J. Trout, and M. J. Beach. 1999. Chlorine disinfection of recreational water for *Cryptosporidium parvum.* Emer. Infec. Dis. 5: 579-584.

Fayer, R., E. J. Lewis, J. M. Trout, T. K. Graczyk, M. C. Jenkins, J. Higgins, L. Xiao, and A. A. Lal. 1999. *Cryptosporidium parvum* detection in oysters from commercial harvesting sites in the Chesapeake Bay. Emer. Infec. Dis. (in press)

Graczyk, T. K., R. Fayer, D. B. Conn and E. J. Lewis. 1999. Evaluation of the recovery of waterborne *Giardia* cysts by freshwater clams and cyst detection in clam tissue. Parasitol. Res. 85:30-34.

Graczyk, T. K., R. Fayer, M. C. Jenkins, J. M. Trout, J. Higgins, E. J. Lewis, and C. A. Farley. 1999. Susceptibility of the Chesapeake Bay to environmental contamination with *Cryptosporidium parvum.* Environ. Res. 78: (in press)

Graczyk, T. K., R. Fayer, E. J. Lewis, J. M. Trout and C. A. Farley. 1999. *Cryptosporidium* oocysts in Bent mussels (*Ischadium recurvum*) in the Chesapeake Bay. Parasitol. Res. 85:518-521.

Jenkins, M. C., J. Trout, C. Murphy, J. A. Harp, J. Higgins, W. Wergin, and R. Fayer. 1999. Cloning and expression of a DNA sequence encoding a 41 kDa *Cryptosporidium parvum* oocyst wall protein. Clin. Diag. Lab. Immunol. (in press)

PART II. PATHOGEN CONTROL DURING SLAUGHTER AND PROCESSING

CONTROL OF PATHOGENIC AND SPOILAGE BACTERIA ON RED MEAT

ARS Contact Persons:
M. Koohmaraie,
G. R. Siragusa,
M. Rivera-Betancourt

CRIS NUMBER: 5438-32000-014
FSIS CODE: I-12
CRIS TERM. DATE: April 2001

Roman L. Hruska U.S. Meat Animal Research Center
Meats Research Unit
Clay Center, Nebraska
Phone: 402-762-4109
Fax: 402-762-4111
E-mail: laster@email.marc.usda.gov

OBJECTIVE A: Study attachment of bacterial pathogens to specific carcass surface tissues and component molecules by fluorescent and luminescent genetic reporter strains of *E. coli* O157:H7 detected by a multilabel counter and confocal scanning laser microscopy (CSLM).

PROGRESS A: As reported last year, the reporter strains of *E. coli* O157 have been developed and shown to exhibit phenotypic characteristics that are identical to the parent strain of *E. coli* O157. The fluorescent strain is a transformant of the plasmid pBAD-GFP_{uv} that fluoresces based on expression of the green fluorescent protein (GFP) and is detectable with a Victor microplate multi-label counter. The bioluminescent strain is a transformant bearing the plasmid pCGLS1, which emits a constitutive signal of light emission detectable in Victor counter. To date we have demonstrated that light emission from inoculated tissues is detachable with the Victor instrument. Also, washing steps can be monitored for decreases in luminescence. We are evaluating this loss of light signal with concomitant plate counts of the reporter strain. The fluorescent labeled *E. coli* will be used in studies to quantitate attachment to various defined substrata including collagen, fatty tissues, and ground beef components. The fluorescent studies will be conducted on membrane bound and microtiter plate well bound tissue components. We have conducted two sets of experiments using confocal scanning laser microscopy (U. Nebraska Microscopy Center) to study the attachment and detachment of GFP labeled *E. coli* O157 to beef lean and fat surface tissues. We have attempted to correlate the microscopic counts of cells per unit area to traditional plate counts. This experiment also examined the efficacy of carcass washing (water and 2% acetic acid) and stomaching to remove bacteria from the carcass matrix. We have collected two sets of large computer CSLM images and are still in the process of compiling these images for quantitative analysis and interpretation.

IMPACT/TECH TRANSFER A: This information will be the first of its kind to understand the bacterial attachment/detachment or inactivation-on-tissue process in mechanistic terms. This series of experiments will provide data on the contribution of individual carcass tissue components that affect attachment/detachment to the carcass surfaces prior to further processing and even through grinding. Collectively this information will be applied toward designing or testing better methods to process carcasses and preserve the resulting meat.

OBJECTIVE B: Evaluate combinations of multiple antimicrobial interventions on beef and pork trim for affect on survival of pathogen and the resulting microbial profile of the product.

PROGRESS B: Phase one of this project (the construction of a pilot-plant facility to apply antimicrobials to trim meat that simulates the industry) has been completed and this facility has been used for non-pathogen inoculation studies described. We have successfully designed and evaluated a multi-hurdle approach to decontaminating beef trim (Phase two). Our approach was to design a process capable of reducing the coliform count per unit surface area by 3-$\log_{10}$ units per cm^2. This process was to be based on combinations of three antimicrobial interventions combined to take advantage of any potential hurdle effect. The interventions tested in combinations were hot air desiccation (4 to 6 seconds at 950°F), followed by hot water (150° - 180°F) and finished with a 2% lactic acid spray (12-15°C). The microbial profiles of inoculated beef trim tissue (BTT) of lean (BTL) and fat (BTF) were monitored during prolonged refrigerated storage following several antimicrobial treatments. Fresh unaltered bovine feces were used to inoculate BTT prior to all treatments (C, Control; W [water wash for 5 sec/cm]; WL [W + 2% lactic acid spray for 3 sec/cm]; comb1 [W + HW: hot water 150°F for 1 sec/cm + HA: hot air 950°F for 4 sec + L]; comb2 [W + HW: 180°F for 1 sec/cm + HA: 950°F for 5 sec/cm + L]; and comb3 [W + HW: 180°F for 3 sec/cm + HA: 950°F/6 sec + L]). The effects of treatments on bacterial populations were monitored with mesophilic aerobic bacteria (APC), presumptive lactic acid bacteria (PLAB), psychrotrophs (PCT), coliforms, and generic *E. coli* for up to 7 days of storage at 4°C. In the case of BTL, the numbers of APC, PCT, and PLAB were increased during storage at 5°C to 7 days, whereas the numbers of coliforms and *E. coli* remained with not statistically different levels of numbers. For quality aspects of BTL, the treatment of comb1 was determined as the best treatment concerning antimicrobial activity. In the case of the BTF experiment, the reductions by treatments were much stronger than in the lean experiments. The pHs of treated BTF increased much more slowly than the pHs of treated BTL, resulting in further reduction of microflora in BTF. Except C and W treatment, all treated samples with lactic acid showed that the numbers of tested microflora were continuously decreasing. In the quality aspects, comb3 was identified as the best treatment for the antimicrobial mixed intervention. Phase three will evaluate the above interventions (now optimized) for the effects of these processes on the microbial ecology of the resulting ground beef held over several days under refrigeration. Microbial analyses will include those mentioned above. Phase four will focus on treatment of pork trim and the microbial profiles of treated, vacuum packaged and refrigerated pork loins. Subsequent work will address reductions of specific surrogate pathogens (e.g., strains of marked biotype one *E. coli* in place of *E. coli* O157, *Listeria innocua* instead of *L. monocytogenes*) on trim only after modification of the existing chamber.

IMPACT/TECH TRANSFER B: This research will be applicable to reducing the pathogen load of meat trim. This information will be disseminated through usual publication routes. Engineering concerns will be able to utilize this data to design and build trim intervention systems for trim producers (slaughterhouses with fabrication rooms), as well as for trim users (i.e., grinding facilities).

OBJECTIVE C: Determine the contribution of hide fecal soil to the carcass microbial profile.

PROGRESS C: This series of experiments addresses the issue of whether the hides (especially the perianal and inner thigh areas) are a significant source of bacterial contamination to the finished carcass. We wish to determine if the biotype 1 *E. coli* populations on the carcass are related to those on the hide. In phase one we have collected a total of 125 biotype 1 E. coli isolates from beef carcass perianal and inner thigh areas and, from the same animal, the carcass with the hide removed pre-split, post-split but pre-wash and post-wash from a commercial processing plant. PFGE (Pulse Field Gel Electrophoresis) profiles to compare these strains have been made and are still being compared. Based on the information from this phase of the study we will decide whether further strain characterization is needed.

IMPACT/TECH TRANSFER C: Should we find that the perianal hide soil is a significant source of carcass microbial contamination, the resulting information could direct greater emphasis on pre-hide removal and pre-evisceration antimicrobial interventions. Such interventions might be altered hide pattern cutting or possibly washes specific for the affected areas of the hide to be performed post stunning but pre-hide removal.

OBJECTIVE D: Modify and validate the colorimetric limulus amoebocyte lysate assay and test a rapid fluorometric bacterial phosphatase assay for a rapid microbial load gauge for both hot and chilled carcasses and trim.

PROGRESS D: This is a continuation of past objectives to develop rapid tests for microbial contamination. Previously we have demonstrated the relationship between the carcass microbial load (mesophilic aerobic plate count) and the likelihood of isolating biotype 1 *E. coli* from beef carcasses. Accepting this premise we have tested the use of the LAL test (a colorimetric test based on horseshoe crab extract reagents which detect the presence of lipopolysaccharide, LPS from the gram negative bacterial cell wall). In summary, we have discovered that a slightly modified commercially available LAL test can determine the level of microbial contamination of a carcass that is either hot or chilled within 15 minutes of taking a sponge sample. The LAL test correlates well with standard plate counts ($r^2 = 0.80$ to 0.90) of surface swab samples, whether the carcass surface menstruum is predominantly lean fascia or adipose fascia. The LAL test was found to be sensitive down to aerobic bacterial levels as low as approximately $\log_{10} 2$ cfu/cm^2. A second test we are researching for this purpose is a rapid fluorometric bacterial phosphatase assay (BPA), which is

already commercially available but not validated for red meat production. The BPA also requires approximately 15 minutes to perform; however, the BPA is a far simpler test to perform than the LAL. For instance, the BPA reagents are all contained within a freeze dried, pre-measured form, a distinct advantage for use in processing plants.

IMPACT/TECH TRANSFER D: Such an assay provides manufacturers with a tool to gauge carcass contamination within a timeframe to rectify potential problems in the processing line. Additional interventions might be reapplication of organic acids, monitoring wash temperatures or antimicrobial compounds. Additionally, testing of chilled pre-fabricated trim (e.g., combos) from outside sources can be done with the LAL test since it functions on chilled carcass surfaces as well as hot samples.

OBJECTIVE E: Developing a non-destructive method to sample meat trim based on rinsing.

PROGRESS E: This is a new objective. The processing of animal carcasses requires deboning and cutting the tissues into smaller pieces of meat for either grinding or packing. This process of removing muscle tissue from the skeleton requires the most actual contact and handling of any point within the entire process. Trim from a cutting line is packed into a combo (2,000-lb boxes of trim) for shipment or storage). From this point further contamination by animal sources is primarily from transferal. Therefore, developing a means to sample trim before entering the combo could be critical to identifying trim that is heavily contaminated and could serve as a potential reservoir for pathogen dissemination. This project will develop and evaluate the potential for a water rinse to be used as a means for sampling trim. Basically, a piece of trim is removed from the line, placed in a sterile plastic bag, a 100 ml bottle of sterile distilled water is added, the bag shaken for a short time (~ 10-15 seconds), the trim removed and placed back on the line. The resulting sample can be tested for any number of microbial indicators (total bacterial loads, lactic acid bacterial counts, coliforms, etc.), or it can be used to target specific pathogens such as splitting the sample for *Salmonella* and *E. coli* O157 analysis. To date we have demonstrated that rinsing methods are not significantly different than excision methods for recovering surface bacteria from both predominantly lean and from fat trim. In fact, the trend suggests that more bacteria are recovered from the predominantly lean samples by rinsing than by excising and stomaching. The rinse method requires no stomaching and the samples can be taken at pre-determined time points within a production lot. Sampling time is less than 1 minute. The main variable to address is the effect of the trim piece size on recovery. Our experience suggests that an approximately uniform size of trim can easily be obtained by trained plant personnel. Once we optimize the method in the lab, we will seek to test it in actual beef trim producing plants.

IMPACT/TECH TRANSFER E: This method has the potential for use in monitoring of meat production as well as provide a tool for epidemiological field testing for pathogens. Its use should span both the production/processing agricultural sectors as well as the scientific community.

Part II. Pathogen Control During Slaughter and Processing

OBJECTIVE F: Compare detachment of enterohemorrhagic *Escherichia coli* (rough and smooth phenotypes of O157:H7, O26:H11 and O111:H8) and *Salmonella* Typhimurium DT104, from inoculated beef carcass surface following decontamination interventions.

PROGRESS F: Experiments were conducted to determine the immediate effect of water and acid spray washes (2% acetic acid and 2% lactic acid) against a rough (MA6, lacking the O157 antigen) and smooth (ATCC 43895, expressing the O157 antigen) phenotype pathogenic *Escherichia coli* O157:H7 on beef carcass surface tissue (lean and adipose). Pathogenic bacteria were enumerated immediately after treatments and ELISAs were conducted in parallel to confirm the identity of the pathogens using monoclonal antibodies against O157 antigen (13B3), H7 flagella (2B7), and EHEC epitope (19F8). Long term effects of water, hot water, organic acids, or trisodium phosphate (TSP) spray wash against *Salmonella* Typhimurium DT104, *Escherichia coli* O26:H11 and O111:H8 on lean beef carcass surface tissue were tested. *E. coli* O157:H7 strain ATCC 43895 was also tested for comparison. Aerobic and pathogenic bacteria were enumerated immediately after treatments, after 2 days of refrigerated storage, and after up to 35 days of 4°C vacuum-packed storage. The findings of this study indicated that reductions associated with the carcass washing procedures were statistically significant for all strains and species, but that no differences between removal were found whether the strain was rough or smooth, of different *E. coli* serotype or *Salmonella* Typhimurium DT104.

IMPACT/TECH TRANSFER F: This knowledge further substantiates the efficacy of generalized carcass antimicrobial interventions. These data suggest that even within the wide range of natural phenotypic diversity found amongst bacterial contaminants associated with carcasses, the trends and magnitude of reductions from spray washing are generally similar.

PUBLICATIONS:

Cutter, C. N. and G. R. Siragusa. 1998. Incorporation of nisin into a meat binding system to inhibit bacteria on beef surfaces. Lett. Appl. Microbiol. 27:19-23.

Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1998. Bacterial profile of ground beef made from carcass tissue experimentally contaminated with pathogenic and spoilage bacteria before being washed with hot water, alkaline solution, or organic acid then stored at 4 or 12°C. J. Food Prot. 61:1109-1118.

Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1998. Long-term bacterial profile of refrigerated ground beef made from carcass tissue, experimentally contaminated with pathogens and spoilage bacteria after hot water, alkaline, or organic acid washes. J. Food Prot. 61:1615-1622.

Gray, P. C., I. R. Shokair, S. E. Rosenthal, G. C. Tisone, J. S. Wagner, L. D. Rigdon, G. R. Siragusa, and R. J. Heinen. 1998. Distinguishability of biological material by use of ultraviolet multispectral fluorescence. Applied Optics 37:6037-6041.

Siragusa, G. R., W. J. Dorsa, C. N. Cutter, G. L. Bennett, J. E. Keen, and M. Koohmaraie. 1998. The incidence of *Escherichia coli* on beef carcasses and its association with aerobic mesophilic plate count levels during the slaughter process. J. Food Prot. 61:1269-1274.

Cutter, C. N. 1999. Combination spray washes of saponin with water or acetic acid to reduce aerobic and pathogenic bacteria on lean beef surfaces. J. Food Prot. 62:280-283.

Cutter, C. N. 1999. The effectiveness of triclosan-incorporated plastic against bacteria on beef surfaces. J. Food Prot. 62:474-479.

Harhay, G. P. and G. R. Siragusa. 1999. Hydrogen-deuterium exchange and ultraviolet resonance Raman spectroscopy of bacteria in a complex food matrix. J. Rapid Meth. Automat. Microbiol. 7:35-48.

Siragusa, G. R. Statistical validation of the Track-Dilution plating method from ground beef and carcass surface samples. J. Rapid Meth. Automat. Microbiol. (in press)

Siragusa, G. R., C. N. Cutter, and J. L. Willett. 1999. Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. Food Microbiol. 16:229-235.

Siragusa, G. R., K. Nawotka, S. D. Spilman, P. R. Contag, and C. H. Contag. 1999. Real-time monitoring of *Escherichia coli* O157:H7 adherence to beef carcass surface tissues with a bioluminescent reporter. Appl. Environ. Microbiol. 65:1738-1745.

PROCEEDINGS/ABSTRACTS:

Cutter, C. N. and W. J. Dorsa. 1998. Antimicrobial activity of cetylpyridinium chloride washes against pathogenic bacteria on beef surfaces. Proc. Recip. Meat Conf. 51:177.

Siragusa, G. R. 1999. D-saccharic acid negative phenotype in *Escherichia coli* O157 serotype. 99th General Meeting of the American Society for Microbiology. Abstr. P-49, p. 521.

DEVELOP ON-LINE VERIFICATION AND INTERVENTION PROCEDURES FOR HACCP IN SLAUGHTER/PROCESSING SYSTEMS

ARS Contact Persons:
M. Koohmaraie,
E. D. Berry,
G. Barkocy-Gallagher

CRIS NUMBER: 5438-42000-002
FSIS CODE: M2MI01
CRIS TERM. DATE: November 2001

Roman L. Hruska U.S. Meat Animal Research Center
Meats Research Unit
Clay Center, Nebraska
Phone: 402-762-4109
Fax: 402-762-4111
E-mail: laster@E-mail.marc.usda.gov

OBJECTIVE A: Determine if differences in acid resistance and acid adaptation can affect the ability of organic acid spray washes to reduce populations of *E. coli* O157:H7 from beef carcass tissue.

PROGRESS A: Stationary phase acid resistance and the ability to induce acid tolerance were determined for a collection of *E. coli* O157:H7 strains. These results indicate that different *E. coli* O157:H7 strains exhibit a variety of acid resistance characteristics. For some strains, the induction of tolerance to low pH was demonstrated. *E. coli* O157:H7 strains that were categorized as acid resistant, acid sensitive, or that demonstrated inducible acid tolerance (acid adaptable) were used to determine if these differences in acid resistance characteristics can play a role in the efficacy of organic acid spray washing to reduce this pathogen on beef carcass surfaces. Beef carcass tissue was inoculated with bovine feces containing 10^6 CFU/g of acid-adapted or non-acid-adapted *E. coli* O157:H7 and subjected to either a water spray wash or a 2% (vol/vol) acetic acid spray wash. After treatments, tissue was refrigerated at 4°C for 2 d, then vacuum-packaged and held at 4°C for a total of 14 days. For both the acid resistant and acid adaptable strains, higher populations of acid-adapted cells remained on beef tissue following 2% acetic acid treatments as compared to non-acid-adapted cells, and these differences remained throughout 14 days of 4°C storage. For both strains, remaining populations of acid-adapted cells following 2% acetic acid treatments were similar to those of acid-adapted and non-acid-adapted cells on tissue receiving water treatments. These data indicate that adaptation to acidic conditions by *E. coli* O157:H7 can negatively influence the effectiveness of 2% acetic acid spray washing to reduce this organism on carcasses.

IMPACT/TECH TRANSFER A: Organic acid spray washes as antimicrobial treatments for carcasses are in use by many segments of the beef processing industry. Exposure to low pH and organic acids in the bovine gastrointestinal tract may result in the induced acid resistance of *Escherichia coli* O157:H7 and other pathogens that may subsequently contaminate beef carcasses. It is important to confirm that organic acid spray washes are efficacious for the most resistant strains

of this bacterial pathogen and for those *E. coli* O157:H7 exhibiting maximal acid adaptation. These data have prompted additional experiments to determine the variation of acid resistance and acid adaptability among recent bovine isolates of *E. coli* O157:H7 and to determine the acid resistance status of *E. coli* O157:H7 as naturally shed in bovine feces. This information has been disseminated via presentations made to professional and industrial groups and a manuscript is currently in the review process for publication in a professional journal.

OBJECTIVE B: To assess the effects of various levels of normal meat microflora on the growth and survival of *E. coli* O157:H7 on beef carcass tissue.

PROGRESS B: The goal of antimicrobial interventions applied to animal carcasses is to reduce the levels of pathogens that may contaminate the carcasses. However, pathogen reduction strategies currently utilized by the industry are not specific for pathogens; in addition to reducing pathogens, these treatments can reduce the levels of the harmless microflora present on animal carcasses. It has been hypothesized that fresh meats with aerobic plate counts of 10^5 to 10^6 per g are less likely to be vehicles for disease outbreaks than are "cleaner meats" that have resulted from the use of carcass antimicrobial intervention technologies. These hypotheses propose that these high levels of normal beef microflora are likely to out-compete any pathogens that may be present. In this study, bacterial microflora on pre-rigor beef carcass tissue was enriched, harvested, and inoculated onto UV-irradiated pre-rigor beef carcass surface tissue at initial target levels of 10^5, 10^4, 10^3, and 10^2 APC/cm^2. A "no added microflora" control group of UV-irradiated tissue was inoculated with sterile H_2O. A two-strain *E. coli* O157:H7 inoculum, composed of equal cell numbers of two streptomycin-resistant strains, was inoculated onto all tissues to give an initial level of 10^2 CFU of *E. coli* O157:H7/cm^2. After treatments, tissue was refrigerated at 4°C for 2 d. At 2 days, beef tissue from each microflora level was incubated for up to 14 days as follows: (1) 4°C aerobic storage, (2) 4°C vacuum-packaged storage, (3) 12°C aerobic storage, or (4) 12 °C vacuum-packaged storage. These experiments have recently been completed and the data are currently being analyzed. However, preliminary analysis indicates that when held at appropriate storage temperature of 4°C for up to 14 days, either aerobically or vacuum-packaged, *E. coli* O157:H7 did not grow on beef tissue at any microflora level, including the "no added microflora" level. At this temperature, there was no effect of microflora level on either growth or survival of *E. coli* O157:H7.

IMPACT/TECH TRANSFER B: There are numerous examples of competitive inhibition or other bacterial antagonism of pathogens by natural microflora in foods. However, data from this study indicate that antimicrobial carcass interventions that substantially reduce the population of normal meat microflora will not promote the unchecked outgrowth of *E. coli* O157:H7 on the carcass surface when beef is stored at proper refrigeration temperatures. This information will be disseminated via presentations made to professional and industrial groups and a manuscript will be prepared for publication in a professional journal.

OBJECTIVE C: To determine if acid tolerance of acid-adapted *E. coli* O157:H7 on beef tissue is sustained during refrigerated storage of beef.

PROGRESS C: During the course of related experiments, it was observed that populations of both acid-adapted and non-acid-adapted *E. coli* O157:H7 on bovine carcass tissue gradually declined during 4°C storage up to 14 days, when the beef tissue was either spray-washed with water or left untreated prior to storage. In contrast, when tissue was spray-washed with 2% acetic acid prior to 4°C storage, surviving populations of *E. coli* O157:H7 did not change. This indicated a possible cross-protective relationship between acid adaptation and cold temperature. Preliminary examination at this time indicated that some degree of acid tolerance of acid-adapted *E. coli* O157:H7 persisted during refrigerated storage of beef. Additional experiments are planned to specifically examine if acid tolerance of acid-adapted *E. coli* O157:H7 on beef tissue is sustained during refrigerated storage of beef, and if exposure to organic acids enhances the survival of this microorganism during refrigerated storage of beef.

IMPACT/TECH TRANSFER C: Several works have indicated that acid tolerance of *E. coli* O157:H7 is enhanced or maintained longer upon incubation at refrigeration temperatures. It is important to confirm that organic acid spray wash interventions increase the safety and quality of meat, and do not result in the development of acid tolerance or in cross-protective bacterial resistance to further processing or preservation treatments. Results will validate current carcass intervention strategies, or identify potential problem areas where preventative measures can be implemented.

OBJECTIVE D: To determine the acid resistance status of *E. coli* O157:H7 naturally shed in bovine feces.

PROGRESS D: This project is in the start-up phase. A most probable number-ELISA procedure is being developed to enhance the enumeration of both healthy and injured *E. coli* O157:H7 in bovine feces. Cattle shedding *E. coli* O157:H7 will be identified. Initial numbers of *E. coli* O157:H7 and percent survival following exposure to low pH (pH 2.5 for 2 h or more) will be determined using slurries of freshly-shed bovine feces. PFGE patterns from isolates of each fecal sample tested will be obtained and compared to assess uniqueness of isolates. The acid resistance characteristics and ability to induce acid tolerance will be determined *in vitro* for each unique isolate, by exposing the cells to the same acidic conditions. *In vitro* and *in vivo* acid resistance will be compared to assess the relative acid tolerance status of *E. coli* O157:H7 as shed from cattle.

IMPACT/TECH TRANSFER D: Exposure to low pH and organic acids in the bovine gastrointestinal tract may result in the induced acid tolerance of *E. coli* O157:H7 and other foodborne pathogens shed in bovine feces. In addition, bovine feces are a source of bacterial contamination of carcasses, and acid tolerance may affect the efficacy of decontamination procedures. The acid resistance status of this organism as shed from cattle should be determined, in order to confirm that organic acid spray washing as currently practiced is effective against this organism as a fecal contaminant of carcasses.

OBJECTIVE E: Determine the variation of acid resistance and acid adaptability among recent bovine isolates of enterohemorrhagic *E. coli* (EHEC).

PROGRESS E: This project has been initiated. The stationary phase acid resistance characteristics and ability to induce acid tolerance for a large collection of recent livestock EHEC isolates (O157, O26, others) will be determined. The pulsed field gel electrophoresis (PFGE) patterns of many of the O157 isolates have been obtained, and the O157 isolates will be chosen such that different PFGE types are represented. In addition, isolates will be chosen such that different geographic locations are represented. Recent bovine isolates of biotype 1 *E. coli* will be included for comparison to EHEC.

IMPACT/TECH TRANSFER E: It has been demonstrated that inherent acid resistance and adaptation to acidic conditions can affect the ability of 2% acetic acid spray washes to reduce levels of *E. coli* O157:H7 from beef. It is important to know the prevalence of acid resistance and acid adaptability among natural livestock isolates of this bacterium, as well as of other EHEC, in order to validate that organic acid spray washing is efficacious for those EHEC isolates that may to be encountered as beef carcass contaminants.

OBJECTIVE F: To identify genes involved in the development of tolerance to low pH and organic acids by *E. coli* O157:H7, and determine if these genes are expressed upon organic acid spray washing of beef.

PROGRESS F: This project is ongoing. Sampling, RNA isolation, and RT-PCR (reverse transcription-polymerase chain reaction) procedures for studying gene expression of bacteria on beef carcass surfaces were developed and demonstrated. *E. coli* O157:H7 isolates that exhibit inducible acid tolerance to acetic and lactic acids have been identified for use in these studies. Prokaryotic differential display techniques have been adapted and optimized for fluorescent differential display using the Beckman GenomyxLRSC apparatus. When candidate acid adaptation genes have been confirmed, the *in vivo* studies will be initiated. The sequences of identified genes will be used to develop oligonucleotide primers for targeted differential display and nucleic acid probes for ribonuclease protection assays for experiments to determine if these genes are induced or expressed in *E. coli* O157:H7 on beef in response to organic acid spray wash treatments.

IMPACT/TECH TRANSFER F: It is important to confirm that organic acid spray wash interventions increase the safety and quality of meat, and do not result in the development of acid tolerance or in cross-protective bacterial resistance to further processing or preservation treatments, or contribute to the development of increased virulence. These experiments are expected to identify both genes previously described and those not known to be involved in acid adaptation by *E. coli* O157:H7, and will provide greater understanding of the molecular mechanisms of acid tolerance development. Knowledge of these mechanisms may identify targets for antimicrobial interventions for control of acid resistance development. This information will be disseminated via presentations made to professional and industrial groups and by publication in professional journals.

OBJECTIVE G: Determine if horizontal gene transfer can occur between *E. coli* O157:H7 and other intestinal non-enterohemorrhagic bacterial microflora in bovine feces.

PROGRESS G: PCR procedures were developed and validated to detect virulence factors from enterohemorrhagic *E. coli* (EHEC; Stx1, Stx2a, Stx2b, *eae*, *hly*, and *chuA*) from fecal cultures enriched for total enterobacteriaceae. The initial experimental objective was to test the hypothesis that known pathogenicity genes (virulence factors) of EHEC are distributed amongst other non-EHEC intestinal microflora. In experiments completed thus far, bovine fecal samples that were culture negative for *E. coli* O157:H7 and EHEC were not found to be PCR positive for virulence factor genes associated with *E. coli* O157:H7. Future experiments have been designed to answer the question of whether or not horizontal gene transfer can occur in feces between *E. coli* O157:H7 and other intestinal microflora.

IMPACT/TECH TRANSFER G: This research could provide evidence that a reservoir of virulence factor genes, as well as a mechanism for the emergence of new pathogens, exists by virtue of the mobility of genes amongst fecal microflora. The data would have broad implications for detection and intervention strategies aimed at reducing the presence of pathogens on meat.

OBJECTIVE H: Discover genes and gene products unique to *E. coli* O157:H7.

PROGRESS H: A subtraction hybridization approach is being used to determine unique DNA sequences of *E. coli* O157:H7, using several different strains of *E. coli* in subtraction reactions versus *E. coli* O157:H7 isolates. Various subtracter DNA pools have been prepared as well as pools of DNA from *E. coli* O157:H7 isolates. The identification of unique DNA sequences is expected to reveal unique metabolic processes that can be exploited for specific, sensitive detection methods for this organism.

IMPACT/TECH TRANSFER H: Real time methods for detection of *E. coli* O157:H7 are needed to enforce zero tolerance. The genes and gene products identified in this study should facilitate development of rapid, sensitive, specific methods for detection of *E. coli* O157:H7 on meat.

OBJECTIVE I: Determine the efficacy of chilled water washes for the removal of *E. coli* O157:H7 from beef carcass tissue.

PROGRESS I: A preliminary study indicated that low pressure washes with chilled water were no more effective than room temperature water for the removal of *E. coli* O157:H7 from beef tissue. It remains to be seen whether chilled water could be more effective in removal of bacteria from carcasses when used at high pressure.

IMPACT/TECH TRANSFER I: If chilled water spray washes could be effectively used to reduce the pathogen load on carcasses, the energy cost for the industry to cool carcasses could concomitantly be decreased.

OBJECTIVE J: Examine the specific binding of *E. coli* O157:H7 to proteins of the bovine carcass superficial fascia.

PROGRESS J: Specific binding mechanisms may be responsible in part for the retention of bacteria on the carcass despite the use of antimicrobial intervention strategies. Thus far, *E. coli* O157:H7 has been demonstrated to bind to several proteins of the carcass superficial fascia in vitro. Experiments are in progress to determine the specificity of this binding and to identify the proteins, as well as experiments to determine if binding to these proteins is unique to *E. coli* O157:H7. In addition, the role of this specific binding to the attachment and retention of *E. coli* O157:H7 on meat is being examined.

IMPACT/TECH TRANSFER J: Understanding the nature of possible specific interactions of *E. coli* O157:H7 with the external carcass surface may lead to novel and specific intervention strategies for removal of this organism from meat animal carcasses.

PUBLICATIONS:

Berry, E. D., and G. R. Siragusa. 1999. Integration of hydroxyapatite concentration of bacteria and seminested PCR to enhance detection of *Salmonella typhimurium* from ground beef and bovine carcass sponge samples. J. Rapid Meth. Automat. Microbiol. 7:7-23.

ABSTRACTS:

Berry, E. D., and C. N. Cutter. 1999. Effects of differences in acid resistance and acid adaptation of *Escherichia coli* O157:H7 on efficacy of organic acid spray washing of beef carcass tissue. 99th General Meeting of the American Society for Microbiology, Chicago, IL, Abstr. P-82, p. 526.

ENGINEERING INNOVATIONS AND MICRO DEVELOPMENTS TO REDUCE CONTAMINATION OF POULTRY AND EQUIPMENT

ARS Contact Persons:
C.E. Lyon, J.A. Dickens, R.J. Buhr, J.A. Cason, A. Hinton Jr.

Russell Research Center
Poultry Processing and Meat Quality Research Unit
Athens, Georgia
Phone: 706-546-3205
Fax: 706-546-3633
E-mail: adickens@ars.usda.gov

CRIS NUMBER: 6612-41420-003D
FSIS NUMBER: R31MD1-05
CRIS TERM. DATE: October 2001

OBJECTIVE A: Determine whether spraying of lactic acid-producing bacteria on broilers a few days before processing can reduce the numbers of pathogenic bacteria by possibly lowering the surface pH of the skin and feathers.

PROGRESS A: In cooperation with the Poultry Microbiological Safety Research Unit, lactic acid-producing bacteria isolated from broiler skin have been sprayed on broilers one or two days before processing. Control broilers in the same pen were not sprayed. Four repetitions of the experiment have been completed, but no reductions in pathogens or other bacteria of interest have been demonstrated despite some effects on numbers of lactic acid bacteria and pH.

IMPACT/TECH TRANSFER A: This work was aimed at finding an acceptable on-the-farm treatment to reduce the increase in external bacteria that occurs when broilers are transported to the processing plant, but it appears that skin pH is not affected enough to reduce the numbers of undesirable bacteria.

OBJECTIVE B: Determine the effects of feed withdrawal and transportation on numbers of *Campylobacter* bacteria on New York dressed broiler carcasses.

PROGRESS B: Previous work demonstrated that *Campylobacter* numbers on feathered broilers increase sharply during transportation to the processing plant. In cooperation with the Poultry Microbiological Safety Research Unit, broilers from the same house have been sampled at the farm just before feed withdrawal and at the processing plant to determine whether higher exterior levels of *Campylobacter* observed on arrival at the processing plant are still seen on the carcass after scalding and picking. Three repetitions of the experiment have been completed, but data are still being analyzed.

IMPACT/TECH TRANSFER B: This work may suggest ways to reduce the numbers of *Campylobacter* on finished carcasses by changing the technology of feed withdrawal and transportation.

OBJECTIVE C: To determine the effect of feed withdrawal on the physical, chemical, and microbial characteristics of the crops and ceca of turkeys.

PROGRESS C: Research was conducted on the effect of feed withdrawal on the weight, pH, and native microflora of the crops and ceca of turkeys. Results indicated that crop weight decreased significantly and crop pH increased significantly during feed withdrawal. The number of aerobic bacteria and *Enterobacteriaceae* in the crop increased while the number of lactic acid bacteria in the crop decreased during 24 h of feed withdrawal. In the ceca, there were no significant changes in the weight or pH after turkeys were subjected to 24 hours of feed withdrawal. There were decreases in the cecal lactic acid bacteria and increases in the cecal aerobes and Enterobacteriaceae in the turkeys subjected to feed withdrawal.

IMPACT/TECH. TRANSFER C: This research can be used by researchers, equipment manufacturers, and processors in their equipment design, quality control, and product hygiene programs.

OBJECTIVE D: To determine the effect of feed withdrawal on the concentration of metabolic intermediates in the crop of poultry subjected to feed withdrawal.

PROGRESS D: Research was conducted to determine the effect of feed withdrawal on the concentration of metabolic intermediates in the crop of broilers and turkeys. Findings indicate that there were significantly lower concentrations of acetic, butyric, lactic, and valeric acid in the crops of turkeys and broilers subjected to12 or 24 h of feed withdrawal than in the crops of the animals that were not subjected to feed withdrawal. Feed withdrawal produced a 20 to 100% reduction in the concentration of these acids in the crop. Additionally, there were significantly lower concentrations of propionic acid in the crops of broilers subjected to 12 or 24 h of feed withdrawal than in broilers not subjected to feed withdrawal. Feed withdrawal produced an 81 to 100% reduction in the concentration of propionic acid in the crop of broilers; however, there was no significant difference in the concentration of propionic acid in the crops of turkeys that had been subjected to feed withdrawal and turkeys that remained on feed.

IMPACT/TECH. TRANSFER D: This basic research will assist researchers in understanding the metabolic changes in the crops of poultry. A better understanding will assist researchers in developing strategies incorporating feed withdrawal and treatments pre and post feed withdrawal to reduce or eliminate the enteric pathogens associated with the contents of the crop.

OBJECTIVE E: To determine the effect of liquid cocktail on bacteria in the alimentary tract of broilers subjected to feed withdrawal.

PROGRESS E: Experiments were conducted in which broilers were challenged with *Salmonella typhimurium* bacteria and subjected to feed withdrawal. Broilers were provided water or the cocktail while they were denied access to feed. *Salmonella* were recovered from the crops of broilers that were provided water during feed withdrawal, but no *Salmonella* were recovered from the crops of broilers that were provided the cocktail. There were also significantly fewer Enterobacteriaceae (e.g. *Escherichia coli*) recovered from the crops of broilers provided the cocktail during feed withdrawal than from the crops of broilers that were not provided the cocktail.

IMPACT/TECH. TRANSFER E: A patent disclosure has been filed with the U. S. Patent office and currently efforts are under way seeking a CRADA partner to assist in further development and licencing of the product.

OBJECTIVE F: Silver Ion solution in poultry water during feed withdrawal

PROGRESS F: A silver ion solution was added to the water of broiler type chickens during feed withdrawal to evaluate its effectiveness in reducing pathogens in the crop prior to processing. Broilers were challenged with *Salmonella* Typhimurium prior to feed withdrawal, and at the time of feed withdrawal the birds had free access to either water or the silver ion solution. At the end of the feed withdrawal time birds were cooped and held for 8 hours before processing. The birds were processed and the crops and crop contents analyzed for the *Salmonella.* The crops of the birds on water were found to be positive with the *Salmonella*, whereas the crops of the birds on the silver ion solution were found not to harbor any of the *Salmonella.* Work is continuing with larger studies and will be extended to *Campylobacter* as well.

IMPACT/TECH TRANSFER F: This work is being done under a Trust agreement. If future data continues the same trend as the preliminary data, this could be an economical means of reducing the contamination of carcasses by the pathogen associated with ingesta contamination. This could also be a critical control point in the overall HACCP program of poultry integrators.

OBJECTIVE G: Evaluate solid and wire-mesh transportation flooring on broiler carcass bacteria load prior to and after defeathering.

PROGRESS G: Experiments compared conventional solid to elevated wire mesh flooring during transport and holding of broilers prior to slaughter. External carcass rinses were obtained twice from each broiler, before scalding and defeathering and again after defeathering and removal of the head and feet. *Campylobacter* counts were less for defeathered than feathered carcasses from the solid flooring treatment but did not significantly decrease following defeathering of carcasses from the wire flooring. The incidence of *Campylobacter*-positive carcasses was reduced following defeathering for both flooring treatments, but the percentage of salmonellae-positive carcasses remained constant. Coliform ($\log_{10}$ 6.20 vs 5.63 cfu/mL of rinse) and *E. coli* ($\log_0$ 5.93 vs 5.36) counts in the feathered rinses were higher for the solid flooring compared with wire flooring, respectively, but after defeathering, the number of coliforms ($\log_{10}$ 3.12) and *E. coli* ($\log_0$ 2.91) recovered did not differ between flooring treatments. Aerobic plate count, *Campylobacter* count,

the incidence of *Campylobacter*-positive and salmonellae-positive carcasses for both feathered and defeathered rinses did not differ between flooring treatments.

IMPACT/TECH TRANSFER G: These results indicate that although broilers transported and held on solid flooring had noticeably dirtier breast feathers and higher coliform and *E. coli* counts prior to scalding and defeathering, bacteria recovery from external carcass rinses did not differ between the solid and wire flooring treatments after defeathering. The processing steps of immersion scalding and defeathering consistently lowered carcass bacteria counts and the incidence of *Campylobacter*-positive but not salmonellae-positive carcasses.

OBJECTIVE H: To determine the effect of oleic acid on bacteria associated with broiler skin.

PROGRESS H: Research was conducted in which skin from processed broiler carcasses was rinsed in solutions of oleic acid. The number of total aerobic bacteria, Enterobacteriaceae, *Campylobacter*, and enterococci on the skin after the oleic acid rinse were determined. Findings indicated that rinsing broiler skin in oleic significantly reduces the number of total aerobes, Enterobacteriaceae, *Campylobacter*, and enterococci associated with the skin.

IMPACT/TECH. TRANSFER H: Work is continuing on this project at the present time. No impact or Tech. Transfer information is available at the present time.

OBJECTIVE I: Compare the relative bacteria load from separate external and internal rinses from the same broiler carcass.

PROGRESS I: A dissection technique was developed to eviscerate carcasses (from the crop to the colon) and permit collection of separate external and internal rinses from each carcass. To test the procedure, one rinse for each carcass was spiked with nalidixic acid-resistant *Salmonella* Typhimurium ($>10^4$ cfu/mL). The nonspiked rinse was collected aseptically from either outside or inside the carcass. The marker *Salmonella* was not detected by direct plating for any carcass (level of detection 10 cfu/mL) indicating a very low level of migration of the marker between the external and internal rinse (>0.1%). After enrichment, however, 33% of nonspiked internal rinses and 92% of nonspiked external rinses contained the marker, indicating a greater rate of leakage of the spiked rinse from inside to outside had occurred. This evisceration-rinse technique was used to compare the positive-incidence of the marker *Salmonella* Typhimurium from broilers that were orally challenged with 10^9 cfu/mL. External and internal rinses were collected, in addition to the crop, cecum, and liver. By direct plating, 0-15% of the external and internal rinses were positive for the marker *Salmonella*, indicating levels of less than 10 cfu/mL. Following enrichment, *Salmonella* was detected in 82% of external carcass rinses, 59% of internal carcass rinses, and 88% of the crops, 59% of the ceca, and 18% of the livers. Positive internal rinses were only obtained from carcasses with a positive external rinse and a positive crop, thereby suggesting the possibility of internal contamination during evisceration.

IMPACT/TECH TRANSFER I: These results are from our pilot plant using individual broilers. However, the results suggest that it is possible to eviscerate externally *Salmonella*-positive carcasses without contaminating the internal cavity. Future sample collection from commercial processing plants will determine if the early processing steps of immersion scalding, defeathering, and evisceration alter the incidence of external and internal salmonellae-positive carcasses. Determination of processing steps as noncontaminating or contaminating will permit direction of intervention procedures where necessary.

OBJECTIVE J: Evaluated alternative methods of crop extraction, processing and production parameters, and quantification of extraction force in broilers.

PROGRESS J: Experiments measured peak extraction force and the incidence of ruptured crops of broilers as influenced by the parameters: age, gender, and extraction direction; electrical stunning voltage (12, 50, and 200 V AC) and postmortem electrical stimulation; feed withdrawal duration; and head removal prior to picking. After adjustment by body weight, peak extraction forces did not differ between males and females, suggesting that gender differences are mainly influenced by body weight. Extraction of the crop toward the head consistently resulted in a higher incidence of intact crops (> 91%) than conventional extraction through the thoracic inlet (59 to 72% intact). Electrical stunning voltage, postmortem electrical stimulation, the duration of feed withdrawal, and removing the head prior to defeathering did not significantly alter peak crop extraction force or the incidence of intact crops.

IMPACT/TECH TRANSFER J: Application of postmortem electrical stimulation or extension of feed withdrawal duration to achieve zero fecal tolerance on the pre-chill carcasses would apparently not influence crop removal force or efficiency. The possibility of removing the crop toward the head would result in a higher incidence of crops extracted intact and thereby prevent potential crop contamination of the thoracic cavity during evisceration. Talks have been initiated with a major equipment manufacturer for the possibility of using the data from this project in developing new techniques for evisceration equipment.

OBJECTIVE K: Determine the numbers of *E. coli* and *Campylobacter* on eviscerated, pre-chill carcass halves.

PROGRESS K: Current processing regulations state that high numbers of *E. coli* on processed carcasses indicate fecal contamination and processing that is out of control. Fecal contamination that occurs exactly on the midline of carcasses should be relatively rare, so carcasses with high levels of *E. coli* should have higher-than-average left-right differences in numbers of fecal bacteria such as *E. coli* and *Campylobacter*. Four repetitions of the experiment have been completed, but carcasses with high levels of *E. coli* have not tended to have high left-right *E. coli* differences, high *Campylobacter* counts, or corresponding *Campylobacter* differences between carcass halves.

IMPACT/TECH TRANSFER K: This work may assist the Food Safety Inspection Service in selecting an appropriate bacterial indicator of fecal contamination.

OBJECTIVE L: Treatment of broiler carcasses with an herbal extract.

PROGRESS L: A commercial herbal extract on a salt carrier was tested in a simulated chiller to determine if it could be used to enhance the microbiological quality of fully processed broiler carcasses. Carcasses were chilled in either a. .5, 1, or 2 % solution of the extract for up to 45 minutes at 1 C then rinsed in tap water before microbiological sampling. Additional carcasses received a standard 45 minute chill in ice water at 1 C. The diluent from the whole carcass rinse was evaluated for total aerobes, Coliforms, *Campylobacter*, and *E coli*. Counts for plant run control carcasses (prior to chill) were 3.7, 2.5, 2.1, and 2.0 log 10 cfu/ml for the respective organisms. The standard chilling treatment significantly reduced counts when compared to plant run controls (2.6, 1.4, 0.7, 0.9). The herbal extract treatment significantly reduced counts even further ($P<.01$) to counts of 0.06, 0.04, 0.01, and 0.00 log 10 cfu/ml for total aerobes, coliforms, *Campylobacter*, and *E. coli* respectively. The 1 and .5% concentrations were just as effective as the 2% concentration on all bacteria with the exception of total aerobes which were significantly reduced but by only 2 Log 10. Detectable levels of the monitored organisms were 1 cell per ml for the *E coli*, coliforms, and total counts and 10 cells per ml for the *Campylobacter*. The microbial counts for the carcasses treated with the herbal extract, in reality, would be considered too low to be detected except for the lowest concentrations. A shelf life study using 0.75% Protecta Two has been initiated but the higher percentages still need to be tested.

IMPACT/TECH. TRANSFER L: A trust was initiated with The Bavaria Corporation to test the herbal extract during processing on the microbiological quality of broiler carcasses. The use of the herbal extract could prove to be an extremely effective intervention step in an overall HACCP plan for commercial processors. The delivery of a safer product to the consumer would be the primary impact and concern for poultry processors. Secondary benefits would be increased exports with the EU banning exports treated with chlorine. This could re-open those markets as well as maintaining the markets of countries that are presently considering joining the EU. Research continues into time, temperature, concentrations, and application techniques to find an economical way to use the extract.

PUBLICATIONS:

Buhr, R.J., J.A. Cason, J.A. Dickens, A. Hinton, Jr. and K.D. Ingram. 1999. Influence of flooring type during transport and holding on bacterial recovery from broiler carcass rinses before and after defeathering. Poultry Sci. (in press)

Cason, J.A., A.D. Whittemore and A.D. Shackelford. 1999. Aerobic bacteria and solids in a three-tank, two-pass, counterflow scalder. Poultry Sci. 78:144-146.

Cason, J.R., R.J. Buhr, J.A. Dickens, M.T. Musgrove and N.J. Stern. 1999. Carcass microbiological quality following intermittent scalding and defeathering of broilers. J. Appl. Poultry Res. (in press)

Cason, J.A. and A.D. Shackelford. 1999. Mixing of dye in a model scald tank. Poultry Sci. (in press).

Dickens, J.A., R.J. Buhr and J.A. Cason. 1999. Subcutaneous temperature profile, skin appearance and picking efficiency of immersion and spray scalded broiler carcasses. Poultry Sci., 78:595-599.

Dickens, J.A., M.E. Berrang, and N.A. Cox. 1999. The effect of an herbal extract on the microbiological quality of broiler carcasses. Poultry Sci. (in press).

PROCEEDINGS/ABSTRACTS:

Buhr, R.J., J.A. Cason, J.A. Dickens, A. Hinton, Jr. and K.D. Ingram. 1999. Influence of flooring type during transport and holding on bacterial recovery from carcass rinses before and after defeathering of broilers. Poultry Sci. 78(suppl 1):140(S184).

Buhr, R.J., M.E. Berrang, J.A. Cason and G.N. Rowland. 1999. Procedure for obtaining separate external and internal carcass rinses from eviscerated broilers. Poultry Sci. 78(suppl 1):22(91).

Buhr, R.J., M.E. Berrang, A. Hinton, Jr., K.D. Ingram and G.N. Rowland. 1999. Separate external and internal carcass rinses from eviscerated broilers orally challenged with Salmonella. Poultry Sci. 78(suppl 1):22(92).

Dickens, J.A. 1999. Pathogen reduction strategies in poultry processing. Proc. Georgia Poultry Conf.

Dickens, J.A. and K.D. Ingram. 1999. The effect of an herbal extract, at a reduced concentration on the microbiological quality of broiler carcasses. Poultry Sci. 78:82.

Hinton, Jr., A., R.J. Buhr and K.D. Ingram. 1999. Changes in the weight, pH, and microflora of the crop of turkeys subjected to feed withdrawal. Poultry Sci. 78:97.

Hinton, Jr., A., R.J. Buhr, M.E. Hume and K.D. Ingram. 1999. Changes in the concentration of metabolic intermediates in the crop of poultry subjected to feed withdrawal. Poultry Sci. 78:27.

Hinton, Jr., A., R.J. Buhr and K.D. Ingram. 1999. Changes in the native microflora, weight, and pH of the ceca of turkeys subjected to feed withdrawal. Proc. 86th IAMFES.

Hinton, A. Jr., R.J. Buhr, and K.D. Ingram. 1998. Feed withdrawal and carcass microbiological counts. Proc. Georgia Poultry Conf. pp. 49-54.

Hinton, A. Jr., R.J. Buhr, and K.D. Ingram. 1999. Changes in the native microflora, weight, and pH, of the ceca of turkeys subjected to feed withdrawal. Proc. 86th IAMFES.

REDUCTION OF BIOFILMS RELATED TO BACTERIAL CONTAMINATION AND PATHOGEN LOAD DURING POULTRY PROCESSING

ARS Contact Persons:	CRIS NUMBER:	6612-41420-006
C.E. Lyon, J.W. Arnold,	FSIS CODE:	R3MC06
M. Berrang, R. Meinersmann	CRIS TERM. DATE:	July 2000

B. Russell Research Center
Poultry Processing & Meat Quality Research Unit
Athens, Georgia
Phone: 706-546-3515
Fax: 706-546-3633
E-mail: glyon@ars.usda.gov

OBJECTIVE A: To study the formation and composition of biofilms on processing plant surfaces.

PROGRESS A: Studies to describe the formation of biofilms and the importance of pathogens within the biofilms in the processing plant environment were continued. Digital aroma technology has been successfully used in previous studies to detect bacterial contamination and classify bacterial spoilage of poultry meat. Poultry meat quality degradation was followed by digital aroma technology at different temperatures and lengths of time in storage. A CRADA with Perkin-Elmer Corporation (PE) was brought to a close. Early detection or the onset of spoilage was not correlated with any specific volatile compounds, although bacterial populations increased in density with higher temperatures and longer storage times.

Research has begun to assess the metabolism of biofilm communities as they develop on poultry meat samples. An understanding of the microbial ecology of biofilm communities associated with poultry products is of importance with respect to pathogenicity and food quality. Knowledge of the metabolic capabilities of these mixed microbial populations will allow more efficient intervention strategies to be developed for use in poultry processing environments. Studies were first conducted to determine the feasibility of using 96-well Biolog GN microtiter plates to assess the metabolic activity of biofilm communities. The method developed in initial investigations was subsequently utilized to assess the substrate utilization profiles of bacterial communities (biofilm) as they developed on chicken meat samples stored for varying time periods at two temperatures commonly used in a poultry processing facility. In poultry processing environments, removal or reduction of the concentration of substrates readily metabolized by bacterial communities (biofilm) which develop on processing equipment may decrease contamination of product by decreasing biofilm formation and persistence.

IMPACT/TECHNOLOGY TRANSFER A: This research responds to the needs of the FSIS regulatory program that sets high priority for food safety concerns. Understanding the conditions conducive to formation of bacterial biofilms will provide information important for successful development of HACCP plans for poultry processing. The digital aroma technology has been

transferred to other scientists, regulatory agency, and industry through invited lectures, presentations at scientific meetings, and publications. The cooperative endeavor with PE was mutually beneficial in that the company and ARS explored new areas of research and developed new methods that can be used in future research and that has been presented at scientific meetings and published. Information regarding metabolism of biofilm communities associated with poultry meat has been transferred to other scientists and industry through publication and presentations at scientific meetings.

OBJECTIVE B: To develop methods of preventing the formation of or removing, biofilms on processing plant surfaces.

PROGRESS B: The need for effective control measures has been addressed by studying the efficacy and mechanisms of potential control agents. Chemical agents include several groups of substances that destroy or limit microbial growth on food surfaces or inanimate objects. Representatives of the major groups, based on their modes of action and uses for currently reducing microbial contamination in plant sanitation, were selected for study. The efficacy of these disinfectants and sanitizers was measured and compared for use in the food industry.

Initial work began under a CRADA between Ajay Corporation and ARS. The primary objective for this study will be to develop means to protect poultry products from microbial contamination that can result in spoilage or pose a threat to food safety. The resistance of a number of bacterial species that are most commonly associated with food-borne illness and food spoilage in the poultry processing environment, including both gram-negative (such as *Salmonella* and *E. coli*) and gram-positive (such as *Listeria monocytogenes* and *Staphylococcus aureus*) was measured in laboratory assays. Future work will include developing more effective formulas for activity against bacterial growth and biofilms in the laboratory and in the poultry processing environment. The ideal product would be non-toxic and have the greatest efficacy against pathogenic bacteria, the least impact on the environment, and the lowest cost for the poultry processing industry.

Stainless steel is the most common material found in the processing plant, and typifies the bacterial attachment process for most other materials. In separate experiments, samples of stainless steel were treated by physical and electrochemical methods, then tested for susceptibility to bacterial attachment, growth, and biofilm formation. After treatment, each of the treated surfaces was less susceptible to bacterial attachment than untreated stainless steel. Comparative measurements of the surface topography correlated with the reduction in bacterial counts. The results of current studies indicate that the analysis of roughness parameters of surface topography by atomic force microscopy can successfully predict the susceptibility or resistance of a surface material to bacterial attachment and biofilm formation. Previously, stainless steel samples that had been electropolished showed significantly fewer bacterial cells and initial biofilm formations than all others tested. However, the electropolishing process can vary among equipment manufacturers. Parameters for the most efficacious process are being defined currently. Studies of other processes other than electropolishing that are necessary for specific types of equipment due to costs or physical specifications are underway.

IMPACT/TECHNOLOGY TRANSFER B: Finding the least amount of treatment necessary to effectively inhibit biofilms will be economical for the industry and consumers as well as reduce the impact of agriculture on the environment. Food safety could be enhanced by increasing the use of materials that do not support growth and attachment of microorganisms while decreasing the use of materials that enhance growth and attachment. Inhibiting bacterial attachment will enhance food safety by preventing the increase in bacterial numbers necessary for biofilm formation. The above studies have provided valuable knowledge about the existence of biofilms and established their importance as an industrial problem. More information is needed to design effective controls. Studying the formation and composition of biofilms on processing equipment surfaces and on food products will establish the basis for efficacious cleaning and sanitizing. Results of the research relative to surface materials have been of interest and shared with conveyor, material handling, and equipment manufacturers. Methodology related to efficacious sanitation practices has been of interest and shared with processors and chemical and equipment manufacturers as well as FSIS personnel.

OBJECTIVE C: Determine the numbers of *Campylobacter* found on broiler carcasses at the six main stages of poultry processing.

PROGRESS C: Broiler processing was subdivided into six main sample sites to examine the microbial profile of the carcasses. These sites included carcasses 1) prior to scald, 2) immediately following scald, 3) immediately following de-feathering, 4) immediately following removal of the viscera, 5) just prior to entering the chill tank, and 6) immediately following chilling. Considering only the numbers recovered from carcass rinse samples prior to scald and those found following chilling, the presence of *Campylobacter* is lowered as the carcass proceeds through the processing plant. However, when the six sites are studied individually it becomes evident that the decrease in *Campylobacter* numbers is not linear. The scalding procedure lowers the population of *Campylobacter* dramatically. However, the numbers rise during the de-feathering operation. Significantly more *Campylobacter* is recovered from carcasses sampled after defeathering than those sampled before (directly after scalding). As carcasses continue to move through the process after de-feathering, the recovery of Campylobacter is lessened.

IMPACT/TECH TRANSFER C: An area of the plant, specifically the picker, has been identified as leading to heightened recovery of *Campylobacter* from broiler carcasses. This information may now be used to help identify a critical control point or suggest application of sanitizer technology that may be used to lower levels of *Campylobacter* on broiler carcasses.

OBJECTIVE D: Measure the levels of *Campylobacter* associated with various organs of broilers as they begin the earliest stages of processing.

PROGRESS D: Experiments were conducted to measure the numbers of *Campylobacter* recovered from five discreet sample sites on broiler carcasses directly after slaughter (prior to scalding and defeathering). Samples included: feathers, skin, crop, ceca and colon. On a per gram basis ceca and

colon had the highest levels of *Campylobacter* with Log_{10} 7.3 and 7.2 cfu per g respectively. Log_{10} 4.7 cfu per g was recovered from the crop, 3.8 from skin and 5.4 from feathers. Rupture or leakage from the ceca or colon represents more of a threat for carcass contamination than the same frequency of leakage from the crop. Regardless of contamination form internal organs the exterior of the carcass carries a substantial number of *Campylobacter* prior to scalding.

IMPACT/TECH TRANSFER D: Levels of *Campylobacter* present in the organs and on the external surfaces of broiler carcasses have been measured. This information can be used to design and maintain effective poultry processing plant sanitation programs.

PUBLICATIONS:

Arnold, J.W. 1999. Advances in food sanitation: Use of intervention strategies. *In:* Current Issues in Food Safety. (in press)

Arnold, J.W. 1999. Early biofilm formation by Salmonella enteritidis. Quant. Microbiol. Cover micrograph, 2 issues.

Arnold, J.W. and S.D. Senter. 1998. Use of digital aroma technology and SPME, GC-MS to compare volatile compounds produced by bacteria isolated from processed poultry. J. Sci. Food Ag. 78:343-348.

Berrang, M.E. and Dickens, J.A. 1999. Presence and level of *Campylobacter* on broiler carcasses throughout the processing plant. J. Appl. Poultry Res. (in press)

Boothe, D.D. Hale, J.W. Arnold, and V. Chew. 1999. Utilization of substrates by bacterial communities (biofilm) as they develop on stored chicken meat samples. Poultry Sci. (in press)

ABSTRACTS:

Arnold, J.W. 1999. Measurements of the surface topography of stainless steel related to bacterial attachment and biofilm formation. Poult. Sci. 78(suppl. 1):82.

Berrang, M.E., R.J. Buhr and J.A. Cason. 1999. *Campylobacter* recovery from the external and internal organs of commercial broiler carcasses prior to scalding. Poultry Sci. 78(suppl. 1):21.

Boothe, D.H. and J.W. Arnold. 1999. Microbial ecology of biofilm communities associated with stored chicken meat samples assessed via substrate utilization patterns. Poultry Sci. 78(suppl. 1):82.

SPECTRAL RADIOMETRY AS AN ON-LINE INSPECTION TOOL

ARS Contact Person:
Y.R. Chen

CRIS NUMBER: 1270-42440-001
FSIS CODE: I-91-6
CRIS TERM. January 2000

Instrumentation and Sensing Laboratory
Beltsville Agricultural Research Center
Beltsville, MD 20705-2350
Phone: 301-504-8450
Fax: 301-504-9466
E-mail: ychen@asrr.arsusda.gov

OBJECTIVE: The overall objective is to develop an automated, real-time system for on-line detection of unwholesome poultry carcasses (as defined by the FSIS) in slaughter plants.

PROGRESS: We have improved the dual-camera imaging system for on-line inspection of poultry carcasses. Two dual-camera systems (one imaging the front of the carcasses, the other the back) were redesigned, fabricated, and evaluated in an in-house pilot scale poultry processing line. Systems level analysis and design integrated individual vision hardware components with image processing algorithms. During pilot-plant testing, the dual-camera imaging system for front carcass inspection delivered accuracies of 91% and 98% for correctively identifying wholesome and unwholesome carcasses, respectively. The back carcass inspection system had 84% and 100% for classification of wholesome and unwholesome carcasses, respectively.

To facilitate the selection of calibration wavelengths and to understand the fundamental spectral features, we used a newly-developed two-dimensional (2D) correlation technique to analyze visible spectra of poultry carcasses of various desease conditions. The results showed that there are at least three prominent visible spectral bands around 445, 485, and 560 nm, which could be attributed to deoxymyoglobin, metmyoglobin, and oxymyoglobin absorption, respectively. The results clearly indicate that there are more variations in oxymyoglobin and deoxymyoglobin and less variation in metmyoglobin in the wholesome and cadaver than in the diseased meats.

Algorithms for identifying individual diseases and defects with the visible/near-infrared system are being developed. A robust, industrial, water-proof poultry inspection system is being assembled and will be installed and tested on-line at a processing plant for a longer period of time.

Working with researchers at the University of Arkansas, Fayetteville, AR, an optical system for inspecting *air sacs* for air sacculitis in poultry carcasses was tested. The experiment showed that the vision method could identify and classify negative and infected *air sacs* with an accuracy of 97%, under laboratory conditions.

A laboratory hyperspectral imaging system for inspection of contamination on foods or agricultural products was developed. The system consists of an image spectrometer, a CCD camera with a control unit, a computer with a frame grabber board, two 150-watt quartz halogen DC line lights, and a conveyor belt with a variable speed control. The imaging spectrometer covers the visible/NIR regions between 430 nm and 900 nm. The 16-bit, 512 x 512 pixel camera is equipped with a back-illuminated CCD detector which has high quantum efficiency up to 90% in the visible region. As the food or agricultural product is moving with the conveyor belt, the imaging spectrometer sequentially scans it line by line to obtain spatial and spectral information of the objects. The conveyor belt is synchronized with the camera's speed for acquiring each frame at a proper spatial resolution.

IMPACT/TECH TRANSFER: A videotape entitled "Automated Chicken Inspection" was prepared with the assistance of the USDA Video, Teleconference & Radio Center. The video describes the operation of ISL's Automated On-line Poultry Inspection System on-line at Tyson Foods, Inc. in New Holland, Pennsylvania. The video has been presented to numerous industry and media groups

PUBLICATIONS:

Chao, K., Y.R. Chen, H. Early, and B. Park. 1999. Color image classification systems for poultry viscera inspection. Appl. Engin. Agric. 15(4):363-369.

Chen, Y.R., and Hruschka, W.R., and Early, H. 1999. A chicken carcass inspection system using visible/near-infrared reflectance: in plant trials. J. Food Proc. Engin. (in press)

Tao,Y., J. Shao, K. Skeeles, and Y.R. Chen. 1999. Detection of splenomegaly in poultry with near-UV and color imaging. Trans. ASAE. (in press)

Watkins, B., Y.C. Lu, and Y.R. Chen. 1999. Economic value and cost of automated on-line poultry inspection for the US broiler industry. Food Control 10: 69-80.

PROCEEDINGS/ABSTRACTS:

Chao, K., and Y.R. Chen. 1998. Color image classification systems for poultry viscera inspection. Proceedings of the International Society for Optical Engineering: Pathogen Detection and Remediation for Safe Eating. 3544:110-120.

Chao, K.B. Park, Y.R. Chen, W.R., Hruschka, and F. W. Wheaton. 1999. Design of a dual-camera system for poultry carcasses inspection. ASAE Paper No. 993118.

Chao, K., Park, B., Chen, Y.R., and W. R. Hruschka. 1999. Chicken heart diseases characterization by multi-spectral imaging. ASAE Paper No. 993117.

Chen, Y.R., W.R. Hruschka, and H. Early. 1998. Online inspection of poultry carcasses using a visible/near-infrared spectrophotometer. Proceedings of the International Society for Optical Engineering: Pathogen Detection and Remediation for Safe Eating. 3544: 146-155.

Ibarra, J.G., Y. Tao, K. Skeeles, L. Newberry, and Y.R. Chen. 1999. Color processing for the identification and classification of air sacculitis in poultry. SPIE Conference on Applications of Digital imaging Processing XXII.

Ibarra, J.G., Y. Tao, K. Skeeles, L. Newberry, and Y.R. Chen. 1999. Neural network color classification of air sacculitis in poultry carcasses. ASAE paper 993157. ASAE Annual Meeting, Toronto, ON.

Liu, Y., Y.R. Chen, and Y. Ozaki. 1999. Characteristization of wholesome and unwholesome chicken meats by two-dimensional visible/near-infrared correlation spectroscopy. ASAE Technical Paper No. 993080. St. Joseph, MI.

Liu, Y., Y.R. Chen, and Y. Ozaki. 1999. 2D Vis/NIR correlation spectroscopy of cooked chicken meats. Proceedings of the International Symposium on Two-Dimensional Correlation Spectroscopy (2DCOS), Kobe-Sanda, Japan, American Institute of Physics. (in press)

Lu, R. and Y.R. Chen. 1998. Hyperspectral imaging for safety inspection of food and agricultural products. Proceedings of the International Society for Optical Engineering: Pathogen Detection and Remediation for Safe Eating. 3544: 121-133.

Park, B., Y.R. Chen, and K. Chao. 1998. Multispectral imaging for detecting contamination in poultry carcasses. Proceedings of the International Society for Optical Engineering: Pathogen Detection and Remediation for Safe Eating. 3544: 156-166.

Tao, Y., J. Shao, K. Skeeles, and Y.R. Chen. 1998. Detection of eviscerated poultry spleen enlargement by machine vision. Proceedings of the International Society for Optical Engineering: Pathogen Detection and Remediation for Safe Eating. 3544: 138-145.

CONTROL OF PATHOGENS TO SURFACES OF POULTRY

ARS Contact Persons:
R. Mandrell, W. Haddon,
R. Binder, D. Brandon,
W. Gaffield

CRIS NUMBER: 5325-41000-021
FSIS CODE: W1MC01
CRIS TERM. DATE: July 2001

Western Regional Research Center
Food Safety and Health
Albany, CA
Phone: 510-559-5998
Fax: 510-559-5818
E-mail: haddon@pw.usda.gov

OBJECTIVE A: Using chemical and physical techniques of surface modeling, identify and describe mechanisms of the attachment of pathogens to surfaces of poultry and of fruits and vegetables.

PROGRESS A: None.

OBJECTIVE B: Using chemical and physical techniques, evaluate the effect of inhibitors and disinfectants on food product surfaces.

PROGRESS B: We are investigating the molecular structure of a possible novel bacterial communication (quorum sensing) molecule in *Campylobacter* that may have implications for pathogen control in food processing. Newly acquired liquid chromatography-mass spectrometry equipment is providing improved data on identification. Improved detection methods for known quorum-sensing molecules are being developed using immunoassay techniques. Evaluation of selected siderophores and siderophore-like compounds are being carried out to elicit the molecular basis by which these bacteria obtain essential nutrients for growth and survival.

IMPACT/TECH TRANSFER B: None

OBJECTIVE C: Develop rapid methods for identifying pathogenic bacteria using laser and thermal desorption mass spectrometry and artificial neural network analysis.

PROGRESS C: A novel detection method based on a technique called proteomics, the measurement of the distribution of proteins produced by an organism, has been applied to both *Campylobacter* and enteropathogenic *E. coli* using laser time-of-flight (MALDI) mass spectrometry as the detection method. Promising preliminary results indicate the ability to distinguish *E. coli* O157:H7 strains from non-virulent strains. Further results on *Campylobacter* using this technique have confirmed

the ruggedness of the method, including a capability to distinguish virulent strains from the non-pathogenic *Campylobacters* and to distinguish mixtures of strains. Through automation, analysis of several hundred samples per day is possible after transfer from culture plates as single colonies.

IMPACT/TECH TRANSFER C: Work reported by us has contributed to widespread interest in this new field for mass spectrometry. A major article in the American Chemical Society publication Today's Chemist in October 1998 gave wide publicity to ARS *Campylobacter* studies. The eventual impact should be a novel rapid, specific method for bacterial analysis using mass spectrometry.

PUBLICATIONS:

Horne, E., T. Coyle, M. O'Keeffe, and D.L. Brandon. Immobilization of thiabendazole-specific antibodies on an agarose matrix for application in immunoaffinity chromatography. Analyst. 124, 87-90. 1999.

ABSTRACTS:

Haddon, W.F., R.E. Mandrell, M.R. Wachtel, W.H. Vensel, and L.A. Harden. 1998. Identification of Food-Borne Bacterial Pathogens Using MALDI-TOF Mass Spectrometry. Pacific Conference on Chemistry and Spectroscopy. San Francisco, Ca.

Brandon, D.L. Immunoassay systems for food safety analysis. 1999 Pacific Southwest Regional AOAC Intl. Annual Meeting. Concord, CA.

ADHESION OF HUMAN PATHOGENS TO SURFACES OF POULTRY

ARS Contact Persons:
R. E. Mandrell
W. Miller

CRIS NUMBER: 5325-41000-022
FSIS CODE: W1MC02
CRIS TERM. DATE: November 1999

Western Regional Research Center
Food Safety and Health
Albany, CA
Phone: 510-559-5829
Fax: 510-559-5948
E-mail: mandrell@pw.usda.gov

OBJECTIVE A: To identify and describe the molecular mechanisms of the attachment of pathogens to surfaces of poultry.

PROGRESS A: Phospholipids, proteoglycans and other proteins have been extracted separately from chicken skin and assayed for binding of *Campylobacter* in a variety of attachment assays. *C. jejuni* binds to specific phospholipids and proteoglycans present in the skin epidermal and dermal layers. Plasmids containing the genes for Green, Yellow and Cyan Fluorescent Protein (GFP, YFP, CFP) have been constucted and optimized for expression in bacteria. Fluorescent strains of *C. jejuni* have been produced and used to identify specific locations of attachment of *Campylobacter* to chicken skin. Mouse monoclonal antibodies (MAbs) that bind specifically to *C. jejuni* and *C. coli* have been characterized further. The antibodies are being used to identify by confocal and scanning electron microscopy *C. jejuni* and *C. coli* bound to poultry tissues. Studies of the inhibition of *Campylobacter* binding to skin are ongoing.

IMPACT/TECH TRANSFER A: US and foreign patents related to anti-*Campylobacter* MAbs have been filed. Eight biotechnology companies evaluated the MAbs for development of detection assays for veterinary, clinical and food inspection use. Three companies have applied for a license. Two additional companies have requested a license application.

OBJECTIVE B: To identify new technology, including new compounds, that can minimize pathogens in foods.

PROGRESS B: Model assays for measuring attachment of *Campylobacter* to poultry have been developed. Approximately 150 different plant essential oils, polyphenolics and plant extracts have been assayed for antimicrobial activity for multiple strains of *C. jejuni*, *Salmonella* and *E. coli* O157:H7. Ten compounds with significant antimicrobial activity have been identified. An assay for measuring antimicrobial activity of compounds for *C. jejuni* as natural contaminants of processed

chicken carcasses and in package water has been developed. Plant antimicrobials with high potency for *C. jejuni* in package water have been identified.

IMPACT/TECH TRANSFER B: Information on the attachment of pathogens in foods (e.g., single organism, biofilms, exterior/interior surface, etc.), and mechanisms of pathogen binding to food surfaces will help producers develop and test strategies to control the amount of viable pathogen contamination and inhibitors of attachment, and/or compounds for controlling the amount of viable pathogen contamination. Plant compounds may provide a non-toxic method for minimizing pathogens in poultry.

PUBLICATIONS:

Mandrell, R.E. and M.R. Wachtel. 1999. Novel detection techniques for human pathogens that contaminate poultry. Current Opinion Biotech. 10:273-278.

ABSTRACTS

Mandrell, R.E., A.H. Bates, A. Charkowski, and G. McDonald. 1999. Attachment of *Campylobacter jejuni* to chicken skin. 99th General Meeting of the American Society of Microbiology, Chicago, IL. Poster P-37.

Charkowski, A., R. Binder, and R. Mandrell. 1999. Characterization of a putative acyl-homoserine lactone from *Campylobacter jejuni*. 99th General Meeting of the American Society of Microbiology, Chicago, IL. Poster P-47.

PATENTS

Mandrell, R.E., A.H. Bates, and D.B. Brandon. 1999. "Monoclonal antibodies against *Campylobacter jejuni* and *Campylobacter coli* outer membrane antigens", US Patent Office Docket No. 0294.97, Serial No. 09/277,599.

ADVANCED TECHNOLOGIES FOR REDUCTION OF MICROORGANISMS AND PARTICULATE MATTER IN FOOD PROCESSING

ARS Contact Persons:
G. H. Robertson, L-S. Tsai,
B. Hernlem, C.H. Huxsoll

CRIS NUMBER: 5325-42000-024
FSIS CODE: W2CC01
CRIS TERM. DATE: July 2002

Western Regional Research Center
Process Chemistry and Engineering Unit
Albany, CA
Phone: 510-559-5878
Fax: 510-559-5777, 5818
E-mail: lstsai@pw.usda.gov

OBJECTIVE A: Develop safe and efficient technologies and systems for disinfecting food process fluids, including methods for the removal of contaminants, and analyzing for disinfectant chemicals.

PROGRESS A: The electroflotation system, developed by our group and reported previously, was evaluated for effectiveness in disinfecting poultry chiller water and model solutions containing *Escherichia coli.* The studies demonstrated that disinfection was effective when chloride was present. The rate of disinfection could be controlled by the manipulation of applied electrical current. While the application of a patent on the invention of a chlorine dioxide/chlorine monitor is pending, the monitor was modified to further improve its sensitivity and reduce body size.

IMPACT/TECH TRANSFER A: Because chloride is almost universally present in food processing water, the electroflotation system is expected to be effective against microorganisms in most food processing water. A patent application for the system is being prepared. Since the release of the development of the chlorine dioxide/chlorine monitor two years ago, several companies have shown interest in developing the device jointly. The modified model with improved specificity and reduced size should greatly increase its commercial adaptability.

OBJECTIVE B: Improve the effectiveness of disinfection and reduce the need of excess chlorine or chlorine dioxide by using gaseous chlorine dioxide for the disinfection of foods.

PROGRESS B: Our previous work has resulted in the approval of aqueous chlorine dioxide by the FDA and USDA as a disinfectant in poultry processing water. This process has gradually been adopted commercially. Aqueous chlorine dioxide was also approved by USEPA to be used to control potato spoilage during storage. Two vendors are testing the process in the northwest region of the US. To minimize the interference of compounds present in the solution and improve disinfection efficiency, an investigation of using gaseous chlorine dioxide on food directly was initiated and carried out. Experimental results showed gaseous chlorine dioxide killed natural flora

and inoculated microorganisms on selected products. However, it also has induced quality changes. Technologies for the use of chlorine dioxide to control microorganisms with minimum quality impact are being sought.

IMPACT/TECH TRANSFER B:

A CRADA was established wit IDI Inc., North Kensington, RI.

PUBLICATIONS: None

PATENTS:

Tsai, L-S., B.J. Hernlem , and C.C. Huxsoll. Determination of concentration of a compound in a multiple component fluid. (Application 12/1998).

QUANTITATIVE DETERMINATION OF PATHOGEN REDUCTION DURING ANIMAL SLAUGHTER AND FOOD PROCESSING TO PROVIDE THE SCIENTIFIC BASIS OF HACCP AND RISK ASSESSMENT

ARS Contact Person	CRIS NUMBER:	1935-42000-034
J.B. Luchansky,	FSIS CODE:	E3IMO1
S. A. Palumbo, M. Medina	CRIS TERM. DATE:	August 2003

Eastern Regional Research Center
Microbial Food Safety Research Unit
Wyndmoor, PA
Phone: (215) 233-6740
Fax: (215) 233-5681
E-mail: spalumbo@arserrc.gov

OBJECTIVE A: Determine the resistance of enterohemorrhagic *Escherichia coli* to sanitizers used in food processing plants.

PROGRESS A: Enterohemorrhagic (EHEC) *Escherichia coli,* including serotype O157:H7, have emerged as significant human pathogens. While contaminated food is a common vehicle, contaminated food processing environments and equipment can also disseminate these bacteria. The resistance/sensitivity of enterohemorrhagic *E. coli* to four sanitizers commonly used in meat plants was investigated using a microtiter plate assay. In addition, since work by other researchers has shown that pretreating bacteria with a stress such as acid can make them more resistant to other stress such as sanitizers, we studied the effect of an acid pretreatment on the resistance/sensitivity of serotype 0157:H7 strains to sanitizers. The sanitizers studied were: Ultrakleen, Per-Ox, Vigilquat, and Chlorine (i.e., NaOCl). Acid pretreatment increased the resistance of the *E.coli* 0157:H7 strains tested to Chlorine and Vigilquat, while the untreated strains were more resistant to Ultrakleen and Per-Ox.

IMPACT/TECH TRANSFER A: These results indicate that sanitizers used against acid-resistant EHEC must be evaluated individually in terms of their effectiveness against strains of EHEC. Additional studies showed that EHEC strains had similar resistance to sanitizers than non-pathogenic strains of *E. coli* and as such, these data suggest that a sanitizing operation can utilize non-pathogenic (i.e., generic) *E. coli* as an indicator of process effectiveness.

OBJECTIVE B: Determine the incidence of *Campylobacter* species in swine slaughter and carcass dressing.

PROGRESS B: *Campylobacter* spp. are fastidious Gram-negative pathogenic bacteria often associated with various animals, including those used for human food. They are also recognized as being difficult to culture and isolate from foods. By combining previously published procedures, *C. jejuni/coli* was readily isolated, detected, and identified on swine carcasses sampled at several steps during their handling sequence. These bacteria were also detected on head meat and from equipment in contact with the swine carcasses.

IMPACT/TECH TRANSFER B: These results indicate that pork can be a vehicle for *Campylobacter* and could ultimately rival poultry as a significant exposure source for humans. Since campylobacteriosis is becoming recognized as a major human foodborne disease agent in the US, these findings can have long term public health significance. In addition, the FSIS may introduce performance standards for *Campylobacter* spp. on red meat, so our research will provide a base line for the incidence of these bacteria on swine carcasses and during carcass dressing.

OBJECTIVE C: Determine the ability of naturally occurring food additives to inhibit the binding of bacterial cells to meat components, including collagen, and evaluate the binding kinetics of *E. coli* O157:H7 and certain of the additives to collagen.

PROGRESS C: Inhibition studies were performed using a BIAcore surface plasmon biosensor. To perform these studies, cells of *E. coli* O157:H7 were bound to the chip surface of the instrument. Binding is indicated by an increase in instrument relative response units (RUs). A group of food and non-food additives was investigated for the ability to reduce binding (cause a decrease in RUs) of a collagen-laminin mixture to *E. coli* O157:H7. The additives varied in the ability to inhibit the binding of the collagen-laminin mixture to *E. coli* O157:H7. Although these additives (identity not possible because of patent disclosure concerns) are used in different food products for other purposes, some also inhibited the binding of *E. coli* O157:H7 to components of meats. Scanning electron microscopy and cell aggregation studies demonstrated that *E. coli* also attached to a purified collagen fiber network. Using meat tissue, it was observed that one specific additive inhibited the binding of *E. coli* O157:H7 to the tissue. Using the BIAcore, young cultures (i.e., 5-6 hrs) showed better binding (i.e., greater increase in RUs) than stationary phase cultures. Studies with the BIAcore and additional additives that had functional properties and chemical structures similar to the ones already studied identified two further compounds which also inhibited (i.e., caused at least an 80% decrease in RUs) the binding of *E. coli* O157:H7 to collagen. In separate experiments, *Salmonella typhimurium* and *S. typhimurium* DT104 were bound to the biosensor chip and the effect of the additives on collagen binding was studied. The same three additives which inhibited binding of *E. coli* O157:H7 to collagen also inhibited binding of *Salmonella* to collagen. The three additives caused an 80% decrease in RUs. From binding studies of the three additives to collagen, the rate of dissociation was 10E-8 to 10E-9 mole for the binding of collagen to the additives compared to 10E-7 to 10E-8 mole for the binding of collagen to *E. coli* O157:H7. The additives that inhibited binding bound more efficiently (i.e., greater affinity constants) to collagen than *E. coli* O157:H7 bound to collagen.

IMPACT/TECH TRANSFER C: These results suggest that food additives with inhibitory activity compete with bacteria for binding sites on the animal carcass. These data are being utilized as supporting evidence for a patent disclosure approved by the ARS in FY99.

OBJECTIVE D: Optimize the detachment of *E. coli* O157:H7 from lean beef skeletal muscle tissue, veal fascia, and connective tissues, and determine the effects of select food additives on bacterial growth.

PROGRESS D: Combinations of selected food grade additives were evaluated for the ability to detach *E. coli* O157:H7 inoculated onto bovine tissue at 10E+6 to 10E+7 CFU/g. About 10E+4 CFU/g remained attached to the tissue and about 10E+3 CFU/g were rinsed off with water. All combinations of additives detached at least 10E+1 to 10E+2 CFU/g of cells. In related experiments, food additives that inhibited binding were evaluated for direct antagonism towards *E. coli* O157:H7, *S. typhimurium,* and *S. typhimurium* DT104; when added to buffered peptone water at 0.3 g/100 ml and incubated at 37°C, these additives had no effect on growth. The level of 0.3g/100ml was chosen to investigate possible bactericidal activity because prefatory binding and detachment studies indicated that this level was the most effective. Also, levels of 0.4g/100ml or above could not be studied because the additives would form gels.

IMPACT/TECH TRANSFER D: These studies provide support for the effectiveness of certain food additives, individually or in combination, to detach *E. coli* O157:H7 from bovine tissue. The filing of US and foreign patents on the types and levels of additives that inhibit binding is anticipated in November/December 1999. Collaboration and a CRADA will be developed with companies and the detachment procedures will be linked to a sampling device and biosensor detection system.

PUBLICATIONS:

Bolton, D.J., A H. Oser, G.J. Cocoma, S.A. Palumbo, and A.J. Miller. 1999. Intergrating HACCP and TQM reduces pork carcass contamination. Food Technol. (4) 53: 40-43.

Palumbo, S.A., A.R. Pickard, and J.E. Call. 1999. Fate of Gram-positive bacteria in reconditioned pork-processing plant water. J. Food Prot. 62: 194-197.

Palumbo, S.A., C. Abeyta, and G. Stelma. 1999. The *Aeromonas hydrophila* group. *In:* Compendium of Methods for the Microbiological Examination of Foods.

Yu, L.S., D. Bolton, C. Laubach, P. Kline, A. Oser, and S. A. Palumbo. Effect of dehairing operations on microbiological quality of swine carcasses. J. Food Prot. (in press)

Tunick, M.H., S.A. Palumbo, and P.M. Fratamico (editors). 1999. New Techniques in the Analysis of Foods. Kluwer Academic/Plenum Publishers, New York.

PROCEEDINGS/ABSTRACTS:

Medina, M.B. 1998. Biosensor studies of collagen and laminin binding with immobilized *Escherichia coli* O157:H7 and inhibition with naturally occurring food additives. Proceedings of SPIE–The International Society for Optical Engineering, pp.97-104.

Medina, M.B, L.W. Tucker and P.H. Cooke. Scanning electron microscopy studies on attachment of *Escherichia coli* O157:H7 to bovine tissues. Presented at 1999 Institute of Food Technologists Annual Meeting, Chicago, IL, July 24-28; Poster at Gordon Research Conference on Molecular Mechanisms of Microbial Adhesions

Palumbo, S.A. and A.R. Pickard. Effect of acid pretreatment on the resistance of enterohemorrhagic *Escherichia coli* to sanitizers. 99th Annual Meeting of the American Society for Microbiology, Chicago, IL.

Palumbo, S.A., J. E. Call, B.S. Marmer, and L.S. Yu. The occurrence of *Campylobacter* spp. in swine carcass dressing operations. 86th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians. Dearborn, MI.

Palumbo, S.A. and L.S. Yu. 1998. The use of *Aeromonas* as a process indicator during swine carcass dressing and cutting. Proceedings of SPIE Conference--The International Society for Optical Engineering, pp.105-108.

Palumbo, S.A., L.S. Yu, and C.E. Briggs. 1999. Identification of motile *Aeromonas* spp. isolated from a swine slaughter plant. 6th International Workshop on *Aeromonas* and *Plesiomonas*. Chicago, IL.

PATENT:

Medina, M.B. Use of naturally occurring food additives in combination with other food chemicals to inhibit attachment of bacterial pathogens to food animal carcasses and also to detach such pathogens from food surfaces. Patent Disclosure (Docket Number 0164.98.) ARS Approved. Anticipated Patent Filing November/December, 1999.

PART III. POST SLAUGHTER PATHOGEN MODELING AND CONTROL

DEVELOPMENT OF MICROBIAL MODELING COMPONENTS FOR USE IN RISK ASSESSMENTS

ARS Contact Persons
J.B. Luchansky
L.L. Zaika

CRIS NUMBER: 1935-42000-032
FSIS CODE: E3MM02
CRIS TERM. DATE: August 2000

Microbial Food Safety Research Unit
Eastern Regional Research Center
Wyndmoor, PA
Phone: 215-233-6655
Fax: 215-233-6581
E-mail: lzaika@arserrc.gov

OBJECTIVE A: Model the effect of temperature and NaCl levels on acid inactivation of *Shigella flexneri* in broth culture.

PROGRESS A: Survival data (lag times, D-values, times to 99.99% inactivation) have been collected for *S. flexneri* in media adjusted to pH 2, 3, 4 and 5 and held at temperatures of 4, 12, 19, 28 and 37°C. Results indicate that the bacterium is capable of survival for extended periods of time and that survival increased with decreasing temperature and increasing pH. Experiments are in progress to assess the effect of NaCl on the survival of the bacterium under the temperature and pH conditions listed above. Data obtained so far involved adjustment of pH with HCl. Experiments have been initiated to determine the effect of adjusting the pH of the growth medium with organic acids normally present or added to foods (e.g., acetic, lactic, citric acid) on survival of *S. flexneri*.

IMPACT/TECH TRANSFER A: These data provide further support for the acid resistance of *S. flexneri* and suggest that acid conditions found in foods may not be sufficient to inactivate this bacterium. This study will provide needed information on the kinetics of non-thermal inactivation of this bacterium. A model will be developed and incorporated into the latest version of the USDA Pathogen Model Program.

OBJECTIVE B: Model the effect of induced pH-dependent stationary phase acid resistance on the survival of *Escherichia coli* O157:H7.

PROGRESS B: The protocol involves the use of four strains of *E. coli* O157:H7, five storage temperatures (37, 28, 19, 12 and 4°C), five pH levels (pH 3, 4, 5, 6 and 7) and four salt (NaCl) levels (0.5, 2.5, 4.5 and 6.5%). Initially, the pH will be adjusted with HCl, and in future experiments, the pH will be adjusted with lactic, malic, acetic, and citric acids.

IMPACT/TECH TRANSFER B: When completed, this study will provide valuable information on the kinetics of acid inactivation of this acid resistant bacterium, particularly in food systems where an acid environment, such as fermented and acidified products, is the major means of assuring microbiological control. A model will be developed and incorporated into the latest version of the USDA Pathogen Model Program.

ABSTRACT:

Zaika, L. L. and J. S. Fanelli. 1999. The effect of temperature on the survival of *Shigella flexneri* at low pH. 86th Int. Assoc. Milk, Food, Environ. Sanitar. Annu. Meet., Dearborn, MI, p-90.

RISK MODELING TO IMPROVE THE MICROBIOLOGICAL SAFETY OF POULTRY PRODUCTS

ARS Contact Persons:
J.B. Luchansky
T. Oscar

CRIS NUMBER: 1935-42000-029
FSIS CODE: E5MA04
CRIS TERM. DATE: February 2000

University of Maryland Eastern Shore
Microbial Food Safety Research Unit
Princess Anne, MD
Phone: 410-651-6062
Fax: 410-651-6568
E-mail: toscar@mail.umes.edu

OBJECTIVE A: Develop predictive models for growth of *Salmonella* spp. on raw and cooked chicken.

PROGRESS A: Developed and validated predictive models for growth of *Salmonella typhimurium* on cooked chicken breast as a function of time, temperature, and previous growth conditions. Established methodology for validating predictive models that involved assessment of model prediction accuracy and bias. Surveyed 12 serotypes of *Salmonella* spp. from chicken ceca for growth kinetics on cooked chicken breast at 25°C. These data indicated only minor differences in growth kinetics among the *Salmonella* spp. tested. Finally, initiated studies with fluorescent *Salmonella* spp. to assess their utility for modeling growth of *Salmonella* spp. on chicken with competing microorganisms.

IMPACT/TECH TRANSFER A: Predictive models were incorporated into the ARS, Poultry Food Assess Risk Model (Poultry FARM) where they can be used by scientists to plan experiments and by consumers to assess the risk and severity of *Salmonella* spp. infections from cooked chicken subjected to temperature abuse.

OBJECTIVE B: Develop simulation models for assessing the risk and severity of human pathogenic bacteria infections from chicken.

PROGRESS B: Developed simulation modeling methods for assessing exposure and response of consumers to human pathogenic bacteria of chicken. Created a farm-to-table model for assessing the risk and severity of *Salmonella* spp. and *Campylobacter jejuni* infections from chicken and created a case study to demonstrate how the model can be used to make food safety decisions.

IMPACT/TECH TRANSFER B: The model was incorporated into Poultry FARM where the chicken industry and FSIS can use it at the processing plant to assess the public health impact of

individual lots of chicken destined for specific distribution channels and consumer populations. Thus far, over 150 copies of Poultry FARM have been distributed to food safety professionals in the U.S. and foreign countries. The primary impact of Poultry FARM has been education of consumers, scientists, regulators, and food producers about the value of computer models as a tool for making food safety decisions.

PUBLICATIONS:

Oscar, T. P. 1998. The development of a risk assessment model for use in the poultry industry. J. Food Safety 18:371-381.

Oscar, T. P. 1999. Response surface models for effects of temperature, pH, and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth. J. Food Prot. 62:106-111.

Oscar, T. P. 1999. Response surface models for effects of temperature and previous temperature on lag time and specific growth rate of *Salmonella typhimurium* on cooked ground chicken breast. J. Food Protect. (in press)

Oscar, T. P. 1999. Response surface models for effect of temperature and previous growth sodium chloride on growth kinetics of *Salmonella typhimurium* on cooked chicken breast. J. Food Protect. (in press)

STRESS ADAPTATION AND VIRULENCE EXPRESSION OF BACTERIAL PATHOGENS IN FOOD ENVIRONMENTS

ARS Contact Persons:
J.B. Luchansky
P.M. Fratamico, D.O. Bayles
S. Bhaduri

CRIS NUMBER: 1935-42000-031
FSIS CODE: E3MM03
CRIS TERM. DATE: February 2003

Microbial Food Safety Research Unit
Eastern Regional Research Center
Wyndmoor, PA
Phone: 215-233-6525
Fax: 215-23-6581
E-mail: pfratamico@arserrc.gov

OBJECTIVE A: Study the effect of cold stress on food-borne pathogens and apply the information to develop technologies to make thermal processing of foods more effective.

PROGRESS A: Previous research showed that a sensitizing cold treatment prior to thermal processing might increase the effectiveness of the thermal process. Cold shocking *Listeria monocytogenes* reduced the subsequent thermal tolerance by up to 37% (decrease in D-value) in foods; changes in the ribosomes correlated with a reduction in the organism's ability to withstand heat. The contamination of fruit juices by bacterial pathogens is currently of considerable interest and concern. Prior cold shock resulted in a reduction in the thermal tolerance of *L. monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella typhimurium* in juices. The D-values of cold shocked *L. monocytogenes*, *E. coli* O157:H7 and S. *typhimurium* were lowered by 27, 26, and 24%, respectively, compared to the corresponding non-cold-shocked cells. The inducible and enhanced vulnerability of *L. monocytogenes*, *Salmonella,* and *E. coli* O157:H7 to heat in model and food systems shows the potential for this approach to be used by the food industry as a practical and efficacious post-processing intervention strategy to eliminate these bacterial pathogens.

IMPACT/TECH TRANSFER A: Cold shock prior to pasteurization of fruit juices could provide an extra measure of safety and would also allow juice processors to reduce thermal processing requirements for pathogen control, while maintaining the fresh qualities of the juice.

OBJECTIVE B: Investigate the effect of stress on the ability to detect and recover low levels of *E. coli* O157:H7 and *Yersinia enterocolitica* from foods and bovine feces, and to study the effect of stress on virulence and other characteristics of *Y. enterocolitica.*

PROGRESS B: Food that undergoes testing for pathogenic bacteria is often subjected to storage temperatures of 4°C or -20°C prior to testing. The food environment also imposes stress on the target

organisms such that detection of low levels of pathogens is often hampered. Pathogenic *Y. enterocolitica* survived freezing in ground pork and milk; however, the organism could not be recovered from samples inoculated at less than 10 CFU/cm^2 of pork using a swabbing technique, whereas it could be recovered from pork samples inoculated at 0.5 CFU/cm^2 that were not subjected to freezing for prolonged periods. *E. coli* O157:H7 was detected by multiplex PCR at a level of ≤1 CFU/g of food (or bovine feces). Similar detection levels were obtained with ground beef samples that underwent enrichment culturing immediately after inoculation and samples that were frozen or refrigerated for 48 h prior to enrichment. The effect of bile salts and other detergents on expression of plasmid, low calcium response, and detergent shock genes, and genes that confer phenotypic and virulence characteristics to *Y. enterocolitica* is also under investigation.

IMPACT/TECH TRANSFER B: The improved detection technologies are being transferred to the FSIS and to industry. Research results will help in assessing the effects of stresses in food environments on assay detection limits and on virulence and other characteristics of bacterial food-borne pathogens.

OBJECTIVE C: Investigate the invasive ability and the tolerance of acid-adapted and non-adapted *S. typhimurium* DT104 to stress conditions.

PROGRESS C: Diarrheal disease caused by *S. typhimurium* DT104 is associated with greater morbidity and mortality than infections caused by other *S. typhimurium* phage types. Thus, a comparison of the ability to invade mammalian cells and of the stress tolerance of *S. typhimurium* DT104 to other *S. typhmurium* strains was performed. Survival of acid-adapted and non-adapted strains in non-pasteurized apple cider, as well as tolerance to hydrogen peroxide, acetic acid, sodium chloride solutions, and to synthetic gastric fluid at pH 2 and pH 3 were determined. The tolerance of *S. typhimurium* DT104 to stresses and the ability to invade mammalian cells were not greater than the stress tolerance and invasive ability of other *Salmonella* evaluated.

IMPACT/TECH TRANSFR C: These studies help in understanding the virulence of *S. typhimurium* DT104, its behavior in foods and response to various stress conditions. The studies indicate that measures taken to inactivate or inhibit the growth of *Salmonella* in food should also be sufficient to inhibit *S. typhimurium* DT104.

PUBLICATIONS:

Bayles, D.O. and B.J. Wilkinson. 1999. Osmoprotectants and cryoprotectants for *Listeria monocytogenes*. Lett. Appl. Microbiol. (in press)

Bhaduri, S. 1998. Isolation of pathogenic *Yersinia enterocolitica* from foods. *In* Recent developments in Microbiology, pp. 139-161. S.G. Pandalai (ed.), Research Signpost, Trivandrum, India.

Briggs, C.E. and P.M. Fratamico. 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob. Agents Chemother. 43:846-849.

Fratamico, P.M., T.P. Strobaugh, M.B. Medina, and A.G. Gehring. 1998. A surface plasmon resonance biosensor for real-time immunologic detection of *Escherichia coli* O157:H7. *In:* New Techniques in the Analysis of Foods, pp. 103-112. M. Tunick, S. Palumbo and P.M. Fratamico (eds.) Kluwer Academic/Plenum Publishers, New York.

PROCEEDINGS/ABSTRACTS:

Bayles, D.O. 1999. Cold shock decreases the thermal tolerance of bacterial pathogens in apple and orange juice. Abstract No. P59, Annual Meeting of the International Association for Milk, Food, and Environmental Sanitarians, Dearborn, Michigan.

Bhaduri, S. and B.J. Cottrell. 1999. Mouse pathogenicity test without iron pretreatment for plasmid-bearing virulent *Yersinia enterocolitica* recovered from pork samples. Abstract No.P-54, Annual Meeting of the American Society for Microbiology, Chicago, Illinois.

Fratamico, P.M. and C.E. Briggs. 1998. Detection and characterization of *Escherichia coli* O157:H7 and *Salmonella typhimurium* DT104, pp. P1-P20. United States-Japan Cooperative Program in Natural Resources - Protein Resources Panel. Honolulu, Hawaii.

Fratamico, P.M., L. Bagi, and T. Pepe. 1999. Rapid detection and identification of *Escherichia coli* O157:H7 in foods and bovine feces by multiplex PCR. Abstract No. P80, Annual Meeting of the Society for Industrial Microbiology, Arlington, Virginia.

ASSURANCE OF MICROBIOLOGICAL SAFETY OF THERMALLY PROCESSED FOODS

ARS Contact Persons:
D. W. Thayer,
V. Juneja, J. Novac

CRIS NUMBER: 1935-42000-028
FSIS CODE: E2MI01
CRIS TERM. DATE: February 2000

Eastern Regional Research Center
Wyndmoor, PA 19038
Phone: 215-233-6500
Fax: 215-233-6406
E-mail: vjuneja@arserrc.gov

OBJECTIVE A: Define the heat treatment required to achieve a specified lethality for *Listeria monocytogenes* in media system to ensure that the heating step is lethal, while avoiding heating that negatively impacts product quality. Use data to develop a mathematical model for determining the effects of environmental parameters on thermal resistance of *L. monocytogenes*.

PROGRESS A: Initial data were collected on the heat resistance of a mixture of *L. monocytogenes* in media system. Environmental parameters assessed were pH (4.0 - 8.0), sodium chloride (0 - 6%), and sodium pyrophosphate (0 - 0.3%). Surviving cells were determined using tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. Decimal reduction times (D-values) were calculated by fitting a survival model to the data with a curve fitting program. The D-values were analyzed by second order response surface regression equation in temperature, pH, sodium chloride and sodium pyrophosphate levels. The four variables interacted to affect the inactivation of the pathogen. Confidence intervals (95%) were developed to allow microbiologists to predict lethality of heat to *L. monocytogenes*. Thermal resistance of *L. monocytogenes* can be lowered by combining these intrinsic factors.

IMPACT/TECH TRANSFER A: The multiple regression equation, developed in this study, can predict D-values for any combinations of temperature, salt, sodium pyrophosphate, and pH that are within the range of those tested. Using this predictive model , food processors should be able to design thermal processes for the production of a safe food with extended shelf life without substantially adversely affecting the quality of the product.

OBJECTIVE B: Develop estimates of parameter values, which characterize a nonlinear survival curve for *Salmonella* spp. subjected to different heating rates in *sous-vide* cooked beef.

PROGRESS B: Inactivation rates of a cocktail of *Salmonella* spp. in *sous vide* cooked beef exposed to varied heating rates of one to three hours from 10°C to the processing temperature of 58°C were determined. From the survival curves, values for two parameters: the asymptotic D-value and the

"lag" time, were estimated. The computations for the nonlinear curves suggested that the treatment affects the initial cell resistance (lag time), but that once this initial period is past, the heat resistance, as measured by the asymptotic D-value; is not affected by the rate of cooking. At 58°C, the asymptotic D-value was estimated to be between 5.2-7.4 minutes. To compute the time to achieve a target lethality (TL), at 58°C, of more than a 4 $\log_{10}$ relative decline, the lag time must be added to the product of TL and the asymptotic D-value.

IMPACT/TECH TRANSFER B: These findings should have substantial practical importance to food processors in *sous vide* cooked beef that are processed by slow heating rate/long come-up times and low heating temperatures. These results allowed food processors and FSIS to predict the results of processing variables and to maintain the safety of processed foods.

OBJECTIVE C: Quantify the heat resistance of *Salmonella* spp. in beef, pork, turkey and chicken containing low, medium and high fat percentages.

PROGRESS C: The heat resistance of eight *Salmonella* serotype cocktails, representing isolates from each species of meat and poultry exhibiting highest heat resistance, was determined at 58 to 65°C in beef (7, 12, 18, 24 and 33% fat), pork (4, 10, 24 and 28% fat), turkey (1, 7, 11 and 13% fat) and chicken (2, 6, 9, 12 and 17% fat). Thermal death times were determined using bags containing meat samples fully submerged in a temperature controlled water bath. The surviving cell population was determined by spiral plating heated samples onto tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. Overall, higher fat levels in all products resulted in higher D-values. D-values (min) at 65C ranged from 0.50-1.13 in beef, 0.50-0.54 in pork, 0.42-0.86 in chicken and 0.55-0.58 in turkey. D-values at 58, 60 and 62.5C were respectively longer. Z-values ranged from 5.58-8.80C. Product composition affected lethality of heat to *Salmonella* spp.

IMPACT/TECH TRANSFER C: Thermal death time values from this study will enable FSIS and processors to predict the effect of fat on the thermal inactivation of *Salmonella* spp. and design acceptance limits on critical control points that ensure safety against the pathogen in cooked ground meat of meat species used in the study.

OBJECTIVE D: Define the heat treatment required to achieve a specified lethality for *Salmonella typhimurium* DT104 in beef.

PROGRESS D: The heat resistance of *Salmonella typhimurium* DT104 was determined at 58 to 65°C in in beef containing 7% 12% 18%, and 24% fat. Thermal death times were determined using bags containing meat samples fully submerged in a temperature controlled water bath. The surviving cell population was determined by spiral plating heated samples onto tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. Inactivation kinetics showed an initial lag period or shoulder before any death occurred with time; higher fat levels in beef resulted in higher lag period. Contaminated beef should be heated to an internal temperature of 58C for 53.5 (7% fat) or

208.1 min (24% fat) to achieve 7-D process for the pathogen. The time at 65C to achieve the same level of reduction should be 7.1 and 20.1 min, respectively.

IMPACT/TECH TRANSFER D: Thermal death time values from this study should assist the food industry in designing HACCP plans to effectively eliminate the pathogen in thermally processed beef with low, medium and high fat percentages. The data were were sent to FSIS for the development of lethality performance standards.

OBJECTIVE E: Determine heat resistance of *Clostridium perfringens* spores produced at 28 to 40C and establish molecular basis of unusually high heat resistance of spores.

PROGRESS E:: Spores of *C. perfringens* enterotoxigenic strains NCTC 8679, NCTC 8238, and H6, were produced at varying temperatures (28, 33, 37, and 40 C) in a modified formulation of Duncan and Strong medium, previously developed in our laboratory. Interestingly, the heat resistance of spores, regardless of sporulation temperature, did not significantly deviate from a mean D-value of 14.75 min. at 100 C. Also, protein profiles obtained from Western immunoblot analyses performed using antiserum raised against sspC, GroEL, GroES, and DnaJ did not show a corresponding change in known stress protein expression as a result of the spore production at different temperatures. Thus, the sporulation temperature was not found to play a role in the acquisition of heat tolerance in spores. However, when *C. perfringens* vegetative cells were exposured for 60 minutes to 28, 37, or 46 C, there was an increase in the expression of specific heat shock proteins GroEL and DnaJ and a parallel increase in the heat resistance of cells was observed. Thus, a transient cause and effect relationship was established.

IMPACT/TECH TRANSFER E: The findings were sent to FSIS to aid in establishing thermal processing guidelines.

PUBLICATIONS:

Juneja, V.K., Whiting, R.C., Marks, H.M. and O.P. Snyder. 1999. Predictive model for growth of *Clostridium perfringens* at temperatures applicable to cooling of cooked meat. Food Microbiol. (in press)

Juneja, V.K., and H.M. Marks. 1999. Proteolytic *Clostridium botulinum* growth at 12 to 48°C simulating the cooling of cooked meat: development of a predictive model. Food Microbiol. (in press)

Juneja, V.K. and Marmer, B.S. 1999. Lethality of heat to *Escherichia coli* O157:H7: D- and z-values determinations in turkey, lamb and pork. Food Res. Int. (in press)

Juneja, V.K. and Eblen, B.S. 1999. Predictive thermal inactivation model for *Listeria monocytogenes* with temperature, pH, NaCl and sodium pyrophosphate as controlling factors. J. Food Protect. (in press)

Juneja, V.K., B.S. Marmer and B.S. Eblen. 1999. Predictive model for the combined effect of temperature, PH, sodium chloride, and sodium pyrophosphate on the heat resistance of *Escherichia coli* O157:H7. J. Food Safety 19:147-160.

IMPROVE MICROBIOLOGICAL SAFETY & SHELF-LIFE OF FOOD BY TREATMENT WITH IONIZING RADIATION

ARS Contact Persons:
D.W. Thayer
C. Sommers, K. Rajkowski

Eastern Regional Research Center
Food Safety Research
Wyndmoor, PA 19038
Phone: 215-233-6582
Fax: 215-233-6406
E-mail: dthayer@arserrc.gov

CRIS NUMBER: 1935-42000-033
FSIS CODE: E2M102
CRIS TERM. DATE: November 2000

OBJECTIVE A: To determine if irradiation pasteurization or modified atmosphere packaging might allow *Listeria monocytogenes* contaminating ground turkey meat following irradiation processing to multiply at faster rates than normal during storage at a mild abuse temperature.

PROGRESS A: *Listeria monocytogenes* did not multiply faster during storage at 7°C on raw ground turkey with a gamma radiation reduced normal flora than on non-irradiated meat, and there was a concentration-dependent inhibition of its multiplication by CO_2. Ground turkey was gamma irradiated at 5°C to 0, 1.5, and 2.5 kGy and inoculated (ca. 100 CFU/g) after irradiation with a cocktail of *L. monocytogenes* ATCC 7644, 15313, 49594, and 43256. The meat was then packaged in air-permeable pouches or under atmospheres containing 30 or 53% CO_2, 19% O_2, and 51 or 24% N_2 and stored at 7°C for up to 28 days. A dose of 2.5 kGy extended the time for the total plate count (TPC) to reach 10^7 CFU/g from 4 to 19 days compared to that for non-irradiated turkey in air-permeable pouches. Following a dose of 2.5 kGy at the end of the 28-day study, the TPCs were $10^{6.42}$ and $10^{4.98}$ under 25% and 50% CO_2 atmospheres, respectively. Under air, 30% CO_2, and 53% CO_2 atmospheres, the populations of *L. monocytogenes* after 19 days incubation were $10^{4.89}$, $10^{3.60}$, and $10^{2.67}$ CFU/g. The populations of lactic acid bacteria and anaerobic or facultative bacteria were also reduced by irradiation. Irradiating ground turkey did not decrease its safety when it was contaminated following processing with *L. monocytogenes*.

IMPACT/TECH TRANSFER A: None at this time, but the results indicate that irradiation will not decrease the safety of a meat if contamination with *L. monocytogenes* occurs following irradiation, that a modified atmosphere containing carbon dioxide will interact with the irradiation treatment to extend shelf-life. These results may be of special importance to the irradiation processing of processed meats. These results have been published in the Journal of Food Protection.

OBJECTIVE B: To determine the combined effects of gamma irradiation and a modified atmosphere in the control or elimination of *L. monocytogenes* on ground turkey during storage at a mild abuse temperature.

PROGRESS B: When sterile ground turkey meat was inoculated with *L. monocytogenes,* packaged under mixtures of nitrogen and carbon dioxide, and irradiated with gamma radiation doses of 0 to 3.0 kGy, there was less bacterial survival in the presence of 100% carbon dioxide than in 100% nitrogen. Radiation resistance was not significantly different in air or in modified atmosphere packaging (MAP) mixtures containing 20, 40, 60, and 80% CO_2:balance N_2. Multiplication of *L. monocytogenes* at 7°C was delayed by a MAP of 50% CO_2 and 50% N_2 compared to aerobic packaging, and irradiated cells decreased in number. The effects of MAP mixtures containing 17.2, 40.5, and 64% CO_2 and nitrogen were compared to aerobic and vacuum-packed turkey inoculated with approximately 5 x 10^3 CFU/g. Samples were irradiated to doses of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 kGy and stored at 7°C for up to 28 days. Irradiation treatments were significantly more lethal in the presence of air than in either vacuum or MAP, and those samples that received > 1.0 kGy there was a concentration-dependent CO_2 inhibition of *L. monocytogenes* multiplication.

IMPACT/TECH TRANSFER B: None at this time, but the results indicate that multiplication of radiation injured *L. monocytogenes* cells is markedly inhibited by the presence of CO_2. This means that the irradiation and MAP interact to achieve an unexpected beneficial inhibition of the growth of this pathogen on meat. The results have been presented at a meeting of the International Association of Milk, Food and Environmental Sanitarians and has been published in the Journal of Food Protection.

OBJECTIVE C: To determine the effect of irradiation temperature on the D-value of *E. coli* O157:H7

PROGRESS C: Processing temperature is known to affect the survival of bacteria during irradiation. Because different methods of cooling may be used during irradiation processing ranging from the use of dry ice to water ice there may be significant differences in the actual temperature of the product during processing. The following study was conducted to allow these effects to be predicted. A mixture of equal amounts of the *E. coli* O157:H7 isolates: ATCC 35150, 43889, 43894, 93-437, and ENT C9490 was used to inoculate radiation sterilized ground beef containing 12.3% fat, 0.89% ash, and 19.7% protein. Samples were gamma irradiated at +20, +12, 0, -4, -12, -20, -30, and -76°C (68 to -104.8°F) to 0, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 kGy. Two independent replications of the study were completed. Radiation D-values were calculated from the results and a response surface equation developed from the data predicting the response at any temperature within this range. The D-values were 0.248, 0.290, 0.388, 0.370, 0.360, 0.606, 0.979, 1.07, 1.11 log CFU/g at +20, +12, +4, 0, -4, -12, -20, -30, and -76°C, respectively. A predictive equation based on these results is under development.

IMPACT/TECH TRANSFER C: None at this time, however the results are expected to be of value both to industry and to regulatory agencies.

OBJECTIVE D: To determine the gamma radiation D-values for *L. monocytogenes* on the surface of commercial frankfurters and to determine if the type of frankfurter influences the resistance of this organism.

PROGRESS D: Frankfurter is a generic term for a cured and cooked sausage, which may consist of almost any meat type and include a wide variety of non-meat fillers and additives. When several "brands" or types of commercially available frankfurters were surface inoculated with *L. monocytogenes* and vacuum packed, gamma radiation D-values ranged from 0.49 to 0.71 kGy, with an average D-value of 0.61 kGy. Differences in gamma radiation D-values were observed for ten of the twenty one pair-wise comparisons by analysis of covariance. Therefore, frankfurter formulation may affect radiation D-value for surface inoculated *L. monocytogenes*. If low dose gamma irradiation were to be used as a process for control of *L. monocytogenes* on frankfurters, gamma radiation dosage should be based on the individual product formulation.

IMPACT/TECH TRANSFER D: None at this time, but the results indicate that treatment with ionizing radiation should provide an effective method for the control of *L. monocytogenes* on frankfurters and possibly other cured meats.

OBJECTIVE E: Determine the effect of ionizing radiation dose-rate (gamma versus electron beam) on the inactivation of *Salmonella typhimurium* Type DT104 on meat.

PROGRESS E: *Salmonella typhimurium* DT104 was inoculated into sterile ground pork and then irradiated using a linear accelerator or a gamma radiation source to determine the D_{10} values. The irradiation was conducted at 3°C, and the composition of the ground pork was either 5%, 50% or 95% fat. There was no significant difference in D_{10} values in the pork, irrespective of fat content. The D_{10} value of the ground pork mixtures averaged 0.39 kGy, which is within the range of irradiation D_{10} values previously reported for *Salmonella* spp. This suggests that *S. typhimurium* DT104 possesses no unique qualities which would predispose it to survival during irradiation processing.

IMPACT/TECH TRANSFER E: None at this time, however the results provide support for the concept that there will be no fundamental differences in the results obtained by gamma versus electron irradiation. These results have been presented at a scientific meeting.

OBJECTIVE F: To determine if there is a significant difference in recovery of stressed cells grown in nutrient limiting reconditioned wastewater.

PROGRESS F: Selective plating media are used for the enumeration and isolation of bacterial pathogens from food and water samples. The study compared the quantitative recovery of *Salmonella* spp. and *Vibrio cholerae* grown in nutrient-limited, filter-sterilized, reconditioned wastewater over the temperature range of 4 to 45°C using nonselective and pathogen-specific selective media. Viable *Salmonella* were enumerated on tryptic soy agar (TSA) and XLT-4, and viable *V. cholerae* were enumerated on TSA and thiosulfate-citrate-bile-sucrose agar. There was a statistically significant (*P*

< 0.05) higher recovery of both pathogens over the growth temperature range on TSA compared to the selective media. Trehalose, a stress-induced metabolite of *Salmonella* was isolated from the cells grown in the reconditioned wastewater, whereas, the *V. cholerae* exhibited a change in cellular morphology from rod to coccoid shape. These results suggest that growth in nutrient-limited water injured or stressed the individual pathogens.

IMPACT/TECH TRANSFER F: Information has been provided by publication to other scientists that indicates that care should be used in selection of plating media for optimum recovery of pathogens from nutrient-limiting environments. These results were presented at the annual meeting of the International Association of Milk, Food and Environmental Sanitarians.

OBJECTIVE G: To determine if *Vibrio parahaemolyticus, Shigella* spp., *V. vulnificus,* and *Pseudomonas aeruginosa* could multiply and/or survive in nutrient limited reconditioned wastewater.

PROGRESS G: We previously reported that *E. coli* O157:H7, *Salmonella* spp., and *V. cholerae* could grow in nutrient-limited, reconditioned wastewater over the temperature range of 4 to 46°C. The BOD of this water was <2 while its AOC was >2. In this current study, we investigated the growth response of *V. parahaemolyticus, Shigella* spp., *V. vulnificus,* and *P. aeruginosa*; both selective and non-selective media were used for their recovery. *V. parahaemolyticus* did not grow or survive at any temperature; *Shigella* spp. Did not grow but survived for >28 days at 4-25°C; *V. vulnificus* did not grow but survived for > 21 days over the entire temperature range; *P. aeruginosa* survived and grew during 14 days at 13-35°C. Non-selective media gave statistically higher recovery than the respective selective media commonly used for these pathogens. These results indicate that caution should be used in attempting direct recoveries of these four Gram negative bacteria from water using selective media.

IMPACT/TECH TRANSFER G: None at this time. These results were presented at the annual meeting of the International Association of Milk, Food and Environmental Sanitarians.

OBJECTIVE H: To determine the effect of gamma radiation on polyethylene terephthalate (PET).

PROGRESS H: A study was initiated to identify volatile and nonvolatile compounds that might be generated during the irradiation of foods packaged in polyethylene terephthalate (PET). Two different semi-rigid PET materials were gamma irradiated to a dose of 25 kGy at room temperature. Volatiles were analyzed using HS/GC/TCD/FID with thermal desorption. The HS/GC/MSD analysis performed at 106°C detected 668-742 ppb formic acid, 862-922 ppb acetic acid, 17-32 ppb 1,3-dioxolane, and 47-71 ppb methyl-dioxolane. Thermal desorption at 200°C detected 10-12 ppm acetaldehyde, 479-975 ppb 1,3-dioxolane, and 6.6-10.6 ppm methyl-dioxolane. The soluble solid extracted from various PET specimens before and after irradiation were in the range of 0.67-0.78%. PET cyclic trimer is the major component and is present at 0.41-0.50%, accounting for more than 50% of the total solids extracted from PET. Irradiation did not increase either the extractable solids

or cyclic trimer. Irradiation had a small effect on the volatile but not the nonvolatile compounds in PET samples.

IMPACT/TECH TRANSFER H: None at this time. The results were present at the annual meeting of the Institute of Food Technologists.

PUBLICATIONS:

Thayer, D.W., E.S. Josephson, A. Brynjolfsson, and G. Giddings. 1998. Uninvited dinner guests. Resource. 5(12):7-8.

Thayer, D.W. and G. Boyd. 1999. Irradiation and modified atmosphere packaging for the control of *Listeria monocytogenes* on turkey meat. J. Food Prot. 62:1136-1142.

Rajkowski, K. T. and R. L. Dudley. 1999. Use of selective media to recover *Salmonella* and *Vibrio cholerae* after growth in reconditioned pork-processing wastewater. J. Food Prot. 62:724-730.

PROCEEDINGS/ABSTRACTS:

Niebuhr, S.E., R.J. Huber, K.T. Rajkowski, D.W. Thayer, and J.S. Dickson. 1999. Sensitivity of *Salmonella typhimurium* DT104 to irradiation. IAMFES Annual Meeting, Dearborn, Michigan. P38, p. 44.

Thayer, D.W. 1999. Control of foodborne pathogens and wholesomeness of irradiated food. Proc. Food Irradiation 99. Washington, DC, Sponsored by Intertech Corporation, Portland, Maine. Paper No. 4, pp 1-19.

Thayer, D.W. and G. Boyd. 1999. Reduction of normal flora by irradiation and its effect on multiplication of *Listeria monocytogenes* on ground turkey at 7°C in a modified atmosphere. IAMFES Annual Meeting, Dearborn, Michigan. P43, p. 45

Rajkowski, K.T. and E. Rice. 1999. Growth and recovery of selected gram negative bacteria in reconditioned wastewater. IAMFES Annual Meeting, Dearborn, Michigan. P88, p. 58.

Komolprasert, V., T.P. McNeal, A. Agrawal, C. Adhikari, and D.W. Thayer. 1999. Volatile and nonvolatile compounds in semi-rigid polyethylene terephthalate materials after 25 kGy gamma irradiation. IFT Annual Meeting, Chicago, IL.

IMPROVE MICROBIOLOGICAL SAFETY & SHELF-LIFE OF FOOD BY TREATMENT WITH IONIZING RADIATION

ARS Contact Persons:
D. W. Thayer
C. Sommers, K. Rajkowski

CRIS NUMBER: 1935-42000-033
FSIS CODE: E2M102
CRIS TERM. DATE: November 2000

Eastern Regional Research Center
Food Safety Research
Wyndmoor, PA 19038
Phone: 215-233-6582
Fax: 215-233-6406
E-mail: dthayer@arserrc.gov

OBJECTIVE A: To determine if gamma irradiation might be a suitable method for the inactivation of *Escherichia coli* O157:H7 and *Salmonella* on alfalfa seed intended for production of sprouts.

PROGRESS A: Ingestion of alfalfa sprouts has been associated with foodborne disease caused by either *E. coli* O157:H7 or *Salmonella.* Radiation sterilized alfalfa seeds were inoculated with mixtures of either non-outbreak strains of *Salmonella* (*S. typhimurium* ATCC 14028, *S. enteritidis* ATCC 13076, *S. senftenberg* ATCC 8400, *S. dublin* ATCC 15480) or outbreak strains (*S. stanley* HO558, *S. newport* H1275, *S. infantis* F4319, *S. anatum* F4317). Sterile alfalfa seeds were inoculated with mixtures of non-outbreak *E. coli* O157:H7 (ATCC 35150, 43889, 43894, 93-937, ENT C9490) or outbreak strains (F4546, SEA 13B88, C7929). The inoculated seeds were exposed to gamma radiation doses from 0 to 2.5 kGy at approximately 0.25 kGy intervals; from the results gamma radiation D-values were determined. Two separate sources of seed were used. There were no significant differences between the D-values for the outbreak and non-outbreak isolates of *Salmonella* spp. nor were there significant differences between the results obtained with the two sources of seed. Water uptake by the seeds during the process of inoculation did not significantly alter the D-value for inactivation of *Salmonella.* The radiation D-values were 0.97 ± 0.03 and 0.60 ± 0.01 kGy for *Salmonella* and *E. coli* O157:H7, respectively. Salmonella were not detectable by enrichment on naturally contaminated alfalfa seed exposed to 1 kGy. The observed D-values for both pathogens are significantly higher than when they are on meat; however, irradiation appears to be a viable treatment process for the inactivation of *E. coli* O157:H7 and suitable in combination with other treatments to inactivate *Salmonella.*

IMPACT/TECH TRANSFER A: None at this time; however, because we are working closely with the International Sprout Growers Association we expect the results to be tested at the industrial level quickly and used when regulatory approval is obtained.

OBJECTIVE B: To determine if sprouts could be treated with ionizing radiation to inactivate *E. coli* O157:H7 or *Salmonella.*

PROGRESS B: In early 1999 there were three reported outbreaks with over 175 cases of salmonellosis associated with the consumption of raw sprouts. Other recent outbreaks were associated with the consumption of sprouts contaminated with *E. coli* O157:H7. Use of ionizing irradiation was investigated as a means to reduce or to completely inactivate these pathogens on sprouts. The radiation D-values for these pathogens were established using a minimum of five doses. The sprouts were pasteurized by irradiation to a dose of 6 kGy before inoculation to inactivate the normal background microflora. The pasteurized sprouts were inoculated with cocktails of either *E. coli* O157:H7 or *Salmonella* spp. made up from vegetable or meat isolates. The radiation D-values for the *Salmonella* spp. were 0.46 and 0.54 kGy for the vegetable and meat isolates, respectively. The radiation D-values for *E. coli* O157:H7 were 0.31 and 0.34 kGy for the vegetable and meat isolates, respectively. *Salmonella* spp. were not detected in naturally contaminated sprouts grown from contaminated seed after treatment with 0.5 kGy. Irradiation did not decrease the quality of the sprouts and extended their shelf-life.

IMPACT/TECH TRANSFER B: None at this time, however we are working cooperatively with the International Sprout Growers Association and plan to evaluate irradiated alfalfa seeds at under commercial conditions.

OBJECTIVE C: To determine if irradiated seed intended for the production of sprouts can be identified from non-irradiated seed.

PROGRESS C: Because ingestion of contaminated sprouts has been linked to several outbreaks of disease it may be necessary to be able to confirm that a given seed supply has been irradiated to inactivate foodborne pathogens. Three methods were evaluated for the identification of irradiated from non-irradiated alfalfa seed: electron paramagnetic resonance (EPR), solid phase micro extraction (SPME) coupled with GC/MS analysis for 1,7 hexadecadiene and 8 heptdecene, and a micro-column procedure followed by GC analysis for hydrocarbons. The EPR procedure requires only 0.1 g of seeds and a few minutes for each analysis. The EPR signal decays rapidly; however, it is possible to identify seeds exposed to 2 kGy four months after irradiation. The SPME procedure requires samples of at least 5 g but is more sensitive and is very stable during storage of the seed. The micro-column procedure requires approximately 100 mg of lipid but requires less sophisticated equipment than the SPME--GC/MS procedure. All three methods were capable of correctly identifying non-irradiated from irradiated (0.5, 1, 2, 3, 4, 5 kGy) alfalfa seeds even after several months of storage.

PROCEEDINGS/ABSTRACTS:

Rajkowski, K.T., D.W. Thayer, and W.F. Fett. 1999. Treatment of contaminated alfalfa seeds by chemicals and/or gamma irradiation to control human pathogens. National Advisory Committee on Microbiological Criteria for Foods (Public Meeting), Washington, D.C.

Part III. Post Slaughter Pathogen Modeling and Control

Thayer, D.W., K.T. Rajkowski. 1999. New developments in irradiation of fresh fruits and vegetables. Institute of Food Technologists Annual Meeting, Chicago, IL.

Rajkowski, K.T. 1999. Keeping quality of sprouts after irradiation and D radiation values of *Salmonella* spp. and *E. coli* O157:H7. 86th International Assoc. Milk, Food & Environmental Sanitarians Meeting. Dearborn, MI.

Rajkowski, K.T., W. Fett, and D.W. Thayer. 1999. Controlling human pathogens on sprouts. International Sprout Growers Association Meeting, New Orleans, LA.

DEVELOPMENT OF MINIMALLY DEGRADATIVE PASTEURIZATION PROCESSES FOR LIQUID OR SOLID FOODS

ARS Contact Persons:
J. Craig, M. Kozempel,
D. Geveke, N. Goldberg,
A. McAloon

CRIS NUMBER: 1935-41420-002
FSIS NUMBER: E1MI01,02
CRIS TERM. DATE: June 2000

Eastern Regional Research Center
Engineering Science Research Unit
Wyndmoor, PA 19038
Phone: 215 233-6589
Fax: 215 233-6795
E-mail: jcraig@arserrc.gov

OBJECTIVE A: Using surface pasteurization, economically reduce microbial contamination on the surface of solid foods (e.g., poultry) without significant loss of product quality.

PROGRESS A: Previous research revealed the VSV (Vacuum/Steam/Vacuum) surface pasteurizer did not treat the visceral cavity as effectively as the outside surface of chicken carcasses. The processor was modified by adding a mandrel assembly which specifically treats the cavity. Using samples inoculated with *Listeria innocua,* bacterial kills were highly significant at 0.7 - 0.8 log cfu/ml using the whole bird rinse sampling procedure. Optimum process parameters were determined with the modified processor using the mandrel assembly. Optimum conditions were; initial vacuum 0.1 s, final vacuum 0.5 s, steam time 0.1 s, and steam temperature 138C. Using chicken "cutlets" at optimum process conditions, bacteria kills were highly significant at 1.0 – 1.3 log cfu/ml *Listeria innocua.* A field surface pasteurizer unit has been designed, components ordered, and assembly is underway.

The VSV surface pasteurization process was applied to hot dogs inoculated on the surface with non-pathogenic *Listeria innocua.* Optimum process conditions were determined for hot dog treatment compatible with current process line speed. Cycling the treatment (repeating the sequence of vacuum/steam/vacuum without exiting the treatment chamber) significantly improved the microbiological kill. At the optimum conditions of steam time of 0.3 s at 138C cycled twice, bacteria kills in excess of 3 log cfu/ml were attained. Pasteurization (killing above 5 log cfu/ml) was reached by increasing to 3 cycles.

IMPACT/TECH TRANSFER A: During the year there were 3 presentations (the Conference on Technology to Improve Food Safety, Washington, DC, the National Turkey Federation Technical and Regulatory meeting, Washington, DC, and the American Meat Institute's meeting "Listeria Interventions for Ready-to-Eat Products", Cincinnati, Ohio). A patent was filed and 2 manuscripts submitted to journals.

Part III. Post Slaughter Pathogen Modeling and Control

Cooperation has been set up with the Poultry Processing and Meat Quality Research Lab, Athens, Georgia to begin field tests in Fall, 1999. Conferred with several equipment manufacturers and negotiations are underway to develop a cooperative research agreement to apply the technology to hot dogs. Cooperation is continuing with Mississippi State University to reduce microorganisms on catfish.

OBJECTIVE B: Develop new electrical pasteurization technology applicable at lower temperatures for temperature sensitive liquid foods such as liquid egg.

PROGRESS B: A Radio Frequency (RF) Dielectric Heater has been developed for isolating thermal and nonthermal effects of RF energy on microorganisms in liquid foods. The modified heater enables the simultaneous application of RF energy and removal of thermal energy from the liquids. A double-pipe heat exchanger is an integral part of the heater. The outer pipe is made of Teflon. The inner pipe is made of stainless steel. Liquid food flows through the annular region between the two concentric pipes and absorbs the RF energy. Concurrently, cooling water flows through the inner pipe and removes the thermal energy from the food, thus controlling the temperature.

IMPACT/TECH TRANSFER B: If, and when, we prove a nonthermal effect exists, develop a process using appropriate organisms, such as molds and yeast in fermentation broth and an *E-Coli* analog in apple cider. Exploit the process by applying it to liquid egg (whole egg, yolk, and egg white). Extend the application of the process to perform novel large-scale organic reactions that produce high yields of pure products without the use of organic solvents or strong acids.

PUBLICATIONS:

Annous, B.A., M.F. Kozempel, and M.J. Kurantz. 1999. Changes in membrane fatty acid composition of *Pediococcus* sp. NRRLB-2354 in response to growth conditions and its effect on thermal resistance. Environ. Microbiol. (in press)

Kozempel, M.F., A. McAloon, and C. F. Yee. 1998. "Pasteurize cider for a penny a gallon". American Fruit Grower, 118(12).

PATENTS:

Kozempel, M.F., N. Goldberg, R. Cook, and M. Dallmer. Non-thermal energy treatment for the reduction of microbial population in liquid food products. U. S. Patent.

RAPID DETECTION OF PATHOGENIC BACTERIA IN FOODS BY BIOSENSORS

ARS Contact Persons:	CRIS NUMBER:	1935-42000-026
Shu-I Tu, P. Irwin, L. Yu	FSIS CODE:	N469009
J. Brewster, C.G. Crawford	CRIS TERM. DATE:	September, 2000

Eastern Regional Research Center
Microbial Biophysics and Biochemistry Research Unit
Wyndmoor, PA 19038
Phone: 215-233-6611
Fax: 215-233-6581
E-mail: stu@arserrc.gov

OBJECTIVES: To develop sensitive, specific and rapid processes that require minimal culture enrichment for the detection of pathogenic bacteria in food systems and to compare with traditional microbial detection and sampling methods.

PROGRESS A: *Optimization of the immunomagentic-electrochemiluminescent (IM-EC) detection of Escherichia coli O157:H7 for practical applications:* We have improved the safety and sensitivity of the immunomagnetic-electrochemiluminescent (IM-ECL) detection of *E. coli* O157 by including a step to heat-kill the pathogen. Heating also releases additional antigenic outer cell membrane material that reacts with the ruthenium labeled reporter antibody thereby increasing the electrochemiluminescence and sensitivity of the assay. We also developed a procedure to confirm the identify *E. coli* O157:H7. Immunomagnetic beads (IMB) specific for the *E. coli* are incubated with an IM-ECL positive sample, then recovered and washed on a paramagnetic column. The column is removed from the magnetic field and the eluted beads are plated on selective media. We have signed a CRADA with IGEN Int'l, who will hire and assign a PhD level scientist to conduct research in our lab, and they will utilize their manufacturing and marketing skills to convert the research assay into a kit, and market the kit to the food industry. *Future Work*: Recent outbreaks of illness have been traced to *E. coli* O111 so we will develop an assay for this pathogen. We will also evaluate the IM-ECL detection of other pathogens and toxins in collaboration with FSIS and IGEN to determine the most effective use of this technology.

IMPACT/TECH TRANSFER A: We have worked with the FSIS Special Projects and Outbreak Support Laboratory to implement the assay into the three FSIS regional Field Laboratories. To date, the assay is being used in the Eastern Field Laboratory and implementation in the other labs should begin soon. Because of our development of the assay for *E. coli*, IGEN has formed a business division to develop and market kits to detect enteric pathogens, non-enteric pathogens (*Listeria, Salmonella, Campylobacter*, etc.) and other organisms such as parasites, mycotoxins, and mycoplasma. They have nearly completed the conversion of the laboratory assay we developed for *E. coli* O157 into a kit and will introduce the kit in October. They have also begun reformatting the

assay into a 96 well format, for their new high-throughput instrument introduced in July, and that format should be available in December.

PROGRESS B. *Determined the factors important to the capture efficiency of immunomagnetic beads in solutions containing low levels of pathogenic bactertia (< 10^3 CFU mL^{-1})*: The capture efficiency of the IMB (diameter = 2.5 mm) specific to *Salmonella* was determined varying both IMB levels (d_{IMB}) and mixing time (t_{mix}) using binomial, 96-well, micro-plate assays. IMB capture efficiency was found to vary with t_{mix} as a pseudo-first order process with a rate constant (k) = 0.028 ± 0.001 min^{-1} and an asymptote of 0.97. Thus, even at extremely low cell densities (d_{cells} ~ 0-70 CFU mL^{-1}), nearly 100% of the bacteria were captured within 2 hours. Based on rather involved physical chemistry treatment, we concluded that cell capture is mainly regulated by IMB mass transport and associated collision probability. In fact, these findings held true even as d_{cells} approached 4 x 10^6 CFU mL^{-1} (d_{IMB} = 8 x 10^6 beads mL^{-1}). Finally, our assumption that every collision resulted in IMB–cell binding appeared reasonable in as much as results from disparate (antibody- [*E. coli* or *Salmonella*] or lectin-based capture mechanisms) systems displayed similar capture kinetics. *Future work*: We will continue the physical chemistry studies of IMB capture to identify the origins of non-specific cell binding using *Proteus* and *E. coli* isolates obtained from poultry samples. We desire to develop a method(s) which allow us to utilize IMBs exclusively as a selective enrichment technique.

IMPACT/TECH TRANSFER B: The results are important for the use of IMB to capture targeted bacteria in solutions. One key finding is that the time needed for complete capture is determined by the size of the beads only. This kinetic consideration is essential for developing standard procedures for using IMB technique in high throughput laboratories.

PROGRESS C. *Developed an ATP-bioluminescence test for detecting viable cells*: We have utilized IMB coated with anti-*E. coli* O157 antibodies to capture *E. coli* O157:H7 in various solutions. The ATP levels of captured bacteria were then determined by the bioluminescence of luciferin/luciferase assay. The cellular ATP content of freshly harvested, IMB-captured E. coli O157:H7 was increased by the addition of glucose but was substantially decreased by the presence of membrane protonophores, e.g., carbonyl cyanide chlorophenyl hydrazone (CCCP). The effects of glucose and CCCP were not observed in the heat-killed or g-ray irradiated *E. coli* O157:H7. The described approach of using the IMB, glucose and CCCP was able to detected 1 CFU of the bacteria per gram of ground beef after a 6-hour enrichment in the EC medium described in **A**. *Future work*: In the coming year, we plan to work together with Contamination Sciences Inc. to optimize the measuring conditions for practical applications. We also plan to expand the above approach to the measurement of *Campylobacter* spp.

IMPACT/TECH TRANSFER C: We have continued the collaborative arrangement with the Contamination Sciences, a company which specializes in bioluminescence measurement, microbead technology and antibody production. Specifically, the company is in the process of developing a

CRADA with us to evaluate and explore the commercialization potential of this bioluminescence detection approach and other methodologies developed by us.

PROGRESS D. *Developed immunoaffinity columns for rapid isolation and concentration of Salmonella.* A method for selectively isolating and concentrating *Salmonella* by a factor of 500 was developed. *Anti-Salmonella* antibody was covalently linked to 40 μ acrylamide particles to prepare a solid phase with affinity for the bacteria. The particles were packed to form small columns, ~1 mm in diameter and 20 μL in volume. Buffer and stomached ground beef containing *Salmonella* at concentrations ranging from 10^2 - 10^6 /mL were pumped through the columns to trap and concentrate the bacteria. Analysis of the incoming and outgoing solution showed that *Salmonella* were trapped in a flow rate dependent manner. At 200 μL/min >98% capture was observed, while at 800 μL/min capture dropped to 10%. In batch experiments using flow at 500 μL/min with recirculation, >90% capture from a 5 mL sample was observed after 40 min. Less than 1% of *E. coli* at 10^5 cells/mL was captured under conditions which gave >98% capture of *Salmonella*, demonstrating the specificity of the technique. By concentrating bacteria from a large volume of sample, this method can help detect pathogens without enrichment. *Future work*: Columns selective for *E. coli* O157:H7 will be characterized and used to isolate this organism from meat and juice products. Integration of this concentration method with a bacteria detection method will be pursued to provide rapid detection of pathogens without enrichment.

IMPACT/TECH TRANSFER D: The developed affinity column method for rapidly concentrating pathogenic bacteria is an attractive alternative to culture enrichment that requires dedicated microbiological facility. Small food producers or processors may consider this acrylamide beads method and the competing magnetic beads technology as a cost-effective mean to achieve microbial food safety requirements.

PROGRESS E. *Improved and Verified Bacteria Detection Using A Dual Filter Immunoelectrochemical Detector.* A limitation of the filtration capture immunoelectrochemical detection method is the direct exposure of the filter to the enzyme-antibody conjugate used to label the bacteria. The conjugate can adsorb to the filter resulting in high background currents. To circumvent this problem, bacteria and conjugate were first captured and concentrated on a primary filter. The flow was then reversed, and the captured bacteria were transferred to a filter/detector. Since the filter/detector was not exposed to conjugate, no background due to adsorption occurred, and background currents were greatly reduced. This work determined the efficiency of transfer and the optimum conditions for transfer. Even at bacteria levels as low as 10/mL transfer efficiency >90% was achieved reproducibly at a flow rate of 100 μL/min using 0.1% Tween buffer. *Future work*: To improve throughput, filtration and labeling will be performed using a 96-sample filtration apparatus coupled to the flow immunoelectrochemical detector. This will reduce analysis time from 30 to 5 minutes per sample.

Impact/Tech Transfer E: The developed dual-filter sample concentration and detection approach represented a significant advancement in minimizing the non-specific background and thus, could substantially increase the reliable pathogen detection limit of immuno electrochemical technology.

PROGRESS F. *Developed two immunomagnetic separation methods for the rapid detection of Campylobacter.* Two immunomagnetic separation methods were developed for the rapid detection of *Campylobacter* spp. in poultry products. Polyclonal anti-*Campylobacter* antibodies conjugated with biotin was used to coat streptavidin-labeled magnetic beads. The same antibodies were also used to coat tosylactivated beads. Coating procedures, incubation time and concentration of the beads affected the sensitivity of the isolation methods. These assays were tested for sensitivity with inoculated ground chicken or turkey and culture suspension. In this approach, which eliminates enrichment, samples were mixed with magnetic beads coated with anti-*Campylobacter* antibodies. The beads, with their bound *Campylobacter*, were magnetically isolated from other sample material and microorganisms. The capture efficiency was determined by plate culture using Karmali medium. We have also applied pulsed-field gel electrophoresis (PFGE) method for gene typing *of Campylobacter* spp. strains recovered from poultry products bought at retail stores. Strain profile of *Campylobacter* will be determined by PFGE to identify the source of the bacterial contamination. *Future work*: We have initiated a collaboration with Kikkoman Corporation, Mundelein, IL., to use the applications of biotinylated firefly luciferase developed by the company. The complexes consisting of *Campylobacter*, biotinylated antibodies and Streptavidin labeled magnetic beads, will be further treated with biotinylated luciferase. Since luciferase is so close to bacterial cells, sensitive bioluminescence detection of ATP in *Campylobacter* can be achieved. Real-time immunological detection of *Campylobacter* using the surface plasmon resonance biosensor of a Biacore instrument is being developed.

IMPACT/TECH TRANSFER F: The described immunomagnetic method, represents a new approach for extracting, concentrating and isolating *Campylobacter* spp. directly from foods without enrichment The developed PFGE process was adopted by a team of ARS scientists working together with Hatfield Meats to identify the sources of *Campylobacter* found in the hog plant located at Hatfield, PA.

PROGRESS G. *Developed a procedure using a light-addressable potentiometric sensor (LAPS) for the detection of E. coli O157:H7 in beef.* We have developed a procedure to rapidly detect the *E. coli* in beef hamburger patties. Beef hamburger spiked with different levels of *E. coli* was then enriched for 5 to 6 h at 37 °C in EC media. Streptavidin coated magnetic beads and biotin-labeled anti *E. coli* O157 antibodies were then added to capture the bacteria. The beads together with captured bacteria were further immobilized on biotinylated 0.45 m nitrocellulose membrane sticks. The bacteria were further treated with fluorescein-labeled anti *E. coli* O157 antibodies and urease conjugated anti fluorescein antibody. Urease catalyzed production rate of NH_3 from urea was then determined by the pH sensitive LAPS of a Threshold Instrument (Molecular Device). The approach allowed us to detect *E. coli* O157:H7 at a level of ~1 CFU/g after a six-hour enrichment. The immobilized bacterial samples were stable in PBS solution for up to 48 h at 4 °C without any

significant change in the LAPS detected signals. This result indicates that the approach may be applied for sample collection from remote locations away from central laboratories equipped with the Threshold Instruments. *Future work*: The developed approach, after proper modifications, will be applied for the detection of *E. coli* O157:H7 in ciders and juices. We also intend to expand the approach for the detection of other pathogenic bacteria, e.g., *Salmonella* and *Campylobacter*, in different food systems.

IMPACT/TECH TRANSFER G: The use of streptavidin coated magnetic beads for linking immuno-captured bacteria to nitrocellulose membrane sticks, increased sample stability for more than 48 h at refrigeration temperature. Thus, it is feasible to transport sample sticks prepared at remote locations to a central laboratory equipped with Threshold instruments for analysis.

PROGRESS H. *Developed simple assay for enumeration of bacteria.* A simple method for non-specific determination of bacteria concentrations in a variety of solid and liquid samples was developed. Samples were combined with media in microwell plates and the turbidity monitored automatically as the bacteria grew in a temperature-controlled plate reader. The time required to reach a threshold turbidity was directly proportional to the log of initial cell concentration. *Salmonella* and *E. coli* concentrations could be determined with a relative error of approximately 20% at levels from 10 to 10^7 cells/mL (with 8 replicates). An assay of 10 samples with standards required approximately 10 minutes to set up and 20 minutes for data processing. The method is used to support many studies on isolation and detection of pathogens in foods. It is more precise and requires much less operator time than other methods such as plate counts and direct counting.

IMPACT/TECH TRANSFER H: None

PUBLICATIONS:

Brewster, J.D. 1999. Automated Filtration Capture Immunoelectrochemical Assay of Bacteria Proc. SPIE 3544: 72-90.

Gehring, A.G., J. D. Brewster, P. L. Irwin, S-I Tu, and L. J. Van Houten, 1999 1-Naphthyl Phosphate as an Enzymatic Substrate for Enzyme-Linked Immunomagnetic Electrochemistry. J. Electro-Anal. Chem. 469: 27-33.

Irwin, P.L., J.N. Brouillette, A.J. Giampa, K.B . Hicks. A.G. Gehring, and S-I. Tu. 1999. Hydration Enthalpy and the Solvent Entropy (Hydrophobic) Effect. Carbohy. Res. (in press)

Mazenko, R.S., F. Riders, and J.D. Brwester. 1999. Filtration Capture Immunoassay for Bacteria: Optimization and Potential for Urine Analysis. J. Microbiol. Meth. 36:157-165.

Sampathkumar, B., I. J. Xavier, L.S. Yu, and G.G. Khachatourians. 1999. Production of listeriolysin O by *Listera monocytogenes* (Scott A) under heat-shock conditions. Int. J. Food Microbiol. 48:131-137.

Tu, S-I, D. Patterson, P. Irwin, and L.S. Yu. 1999. Effects of membrane protonophores on the ATP content of immunomagnetic beads captured *Escherichia coli* O157:H7. J. Rapid Meth. Automation Microbiol. (in press)

Tu, S-I., J. Uknalis, D.L. Patterson, and A. Gehering. 1999. Immunomagnetically Captured, 4',6-Diamidino-2-Phenylindole (DAPI)-Labeled *Escherichia coli* O157:H7 detected by Fluorescent Microscope Imaging. Proc. SPIE . 3544: 32 - 40.

Tu, S-I, J. Uknalis, and A. Gehring. 1999. Detection of Immunomagnetic Bead Captured *Escherichia coli* O157:H7 by Light Addressable Potentiometric Sensor. J. Rapid Meth. Automation. Microbiol. (in press)

PROCEEDINGS/ABSTRACTS:

Irwin, P.L., J. Brewster, W. Damert, S-I. Tu, and A. Gehring, Immuno-magnetic Bead Mass Transport I: Capture Efficiency of Salmonella enteritidis at Low Cell Densities in Phosphate-buffered Saline. (Invited presentation at the Annual Meeting of the Society for Industrial Microbiology,

NEW TECHNOLOGIES TO IMPROVE AND ASSESS MEAT QUALITY AND SAFETY

ARS Contact Persons:	CRIS NUMBER:	1265-41420-001
M.B. Solomon	FSIS CODE:	B3MI03
B.W. Berry, A. M. Campbell	CRIS TERM. DATE:	February 2002

Beltsville Agricultural Research Center
Meat Science Research Lab
Beltsville, MD
Phone: 301-504-8400
Fax: 301-504-8438
E-mail: msolomon@ggpl.arsusda.gov

OBJECTIVE A: Determine the effect of hydrodynamic pressure (HP) on normal spoilage microorganisms found in meats.

PROGRESS A: Hydrodynamic pressure (HP) process uses a high-energy explosive to generate a supersonic shock wave in a liquid medium. The HP process causes major structural alterations in the protein (myofibrillar) structure of meat. This finding led to investigating the effect HP might have on normal spoilage microorganisms found in meats. Fresh (refrigerated @ 5°C) and temperature-abused (room temperature @ 23°C; to increase microbial load) ground beef, whole beef muscle (bottom round), and whole pork muscle (ham) were vacuum packaged and treated with HP. Immediately following treatments, HP samples and controls were assayed for pH and total aerobic plate counts (APC-$\log_{10}$ cfu/g).

IMPACT/TECH TRANSFER A: Statistical analysis showed no significant differences in pH between controls and HP treated meat samples. Initial (pre-HP treatment) bacterial log numbers (APC) were 4.69 for ground beef, 3.61 for whole beef, and 3.58 for whole pork. HP treatment resulted in a significant reduction in APC for fresh meat samples (73% ground beef; 73% whole beef; 34% whole pork) and temperature abused meat samples (69% ground beef; 53% whole beef; 45% whole pork). These results indicate that HP was effective in reducing normal spoilage microorganisms found in fresh and temperature-abused meats and may provide a method to extend the shelf life of refrigerated meats.

OBJECTIVE B: Determine the effect of hydrodynamic pressure (HP) alone or combined with salt on normal spoilage bacteria found in beef.

PROGRESS B: Preserving foods with salt is based on salt having a drying effect on both the food and the microorganisms. Hurdle technology (combining two or more processes to reduce microbial contamination) is being practiced in the meat/food industry to decrease the risk of product recall and product contamination. This study was conducted to determine the effects of HP and salt either alone or in combination on normal spoilage bacteria found on fresh whole beef muscle. Fresh beef

strip loins were cut into steaks. Steaks were treated with either HP, salt (koshering process), or the combination of the two and then stored (vacuum packaged) at 2°C for 14 days. Controls consisted of non-treated samples vacuum packaged and stored at 2°C for 14 days.

IMPACT/TECH TRANSFER B: No differences among treatments were found in muscle pH at either day 0 or day 14. Microbial flora (APC) determination at day 0, immediately after treatment applications, revealed a 42% reduction in microbial flora as a result of treatment applications compared to the controls (log 3.63=Control; 2.11=Salt; 2.12=HP; 2.12=Salt+HP) . Following the 14 day refrigerated storage bacterial numbers increased to a log of 5.0 in the control samples. Salted meat samples increased to log 3.8 after 14 days of storage. HP treated samples remained unchanged (log 2.4) after 14 days of storage while the salt+HP combination treatment resulted in an increase in APC to log 3.2 compared to controls. No lean color differences among treatments were found at day 0, however, at 14 d significant differences due to treatments were observed for both instrumentally and visually appraised lean color stability. Lean surface color visually assessed by a panel found the lean color to be slightly bright cherry-red in color at 0 d for all four treatments, however, at 14 d only the control, HP, and salt+HP treated samples remained slightly bright cherry-red. The salt treated samples were slightly dark cherry red in color. These visual differences were confirmed using instrumental color measurements. No differences among treatments were found for off-odors at either day 0 or day 14.

OBJECTIVE C: Identify mechanisms and develop control procedures to prevent inconsistent cooked color/cooked meat temperature relationships.

PROGRESS C: Joint ARS-FSIS, USDA studies provided convincing evidence that cooked beef patty color is not a good indicator of internal patty temperature. Thus, the new consumer message released by FSIS in 1998 stated "the only way to determine that a ground beef patty has been cooked to a high enough temperature to destroy bacteria is to use a thermometer". Consumers should not eat ground beef patties that are pink or red in the middle unless a food thermometer has been used to verify cooked temperature." However, a number of recent surveys indicate that most consumers either don't own a meat thermometer or if they own one, don't use it in cooking meat products. Thus, additional ancillary studies to the original joint ARS-FSIS study on premature brown color in cooked beef patties, have focused on: (1) the use of outdoor gas grills as the cooking equipment, (2) temperature and color changes post-cooking and before consumption, and (3) use of infrared thermal imagery to assess temperature variability in cooked beef patties. In utilizing the outdoor gas grill for cooking beef patties, with the exception of bulk ground beef frozen and then thawed before making patties, higher levels of fat produced more brown and less pink-red color following cooking. Similar to the findings of the earlier ARS-FSIS project, patties cooked from bulk frozen/thawed product had more brown and less pink-red color than patties cooked from the fresh or thawed state. Termination of cooking by observation of brown color in a slit made in the outer edge of the patty during cooking resulted in internal temperatures of 64.8, 65.7 and 60.4°C for fresh, thawed and bulk-frozen-thawed cooked patties, respectively. Termination of cooking for patties on an outdoor gas grill by observing brown color in slits in the outer edge of patties, does not correspond to a safe internal temperature.

Studies have revealed that the internal temperature of patties continues to increase once they are removed from the cooking environment. However, with thin patties (0.94 cm), the increases in temperature are often not sufficient to guarantee food safety if the patties are removed from the grill between 66.1 and 68.3 °C. Myoglobin continues to denature following removal from the cooking environment during the typical time interval between outdoor cooking and consumption of a hamburger with condiments. Thus, internal color of cooked patties is even less reliable as an indicator of food safety under those conditions.

Use of infrared thermal imagery has revealed the occurrence of considerable temperature variability in cooked beef patties. Ground beef patties were cooked from the fresh, thawed and frozen states to either 57.2, 65.6 or 71.1 °C before assessment of temperature variability on patty cross sections with infrared thermography. Temperature ranges on the cut surfaces often exceeded 18°C. Temperature profiles were higher for patties cooked from the fresh state compared to those cooked from the frozen or thawed states. Fresh patties required longer cooking times to reach targeted temperature endpoints, which may have contributed to their higher temperature profiles. The temperature variability revealed in this study indicates the need to develop methods for controlling temperature variability in patties in order to utilize the new recommendations for use of endpoint temperature as the indicator of food safety.

IMPACT/TECH TRANSFER C: As a result of the new issuance of recommendations by FSIS to use a thermometer to insure beef patties have been cooked to 71°C, FSIS sponsored a thermometer workshop in Washington, DC in November, 1998. The workshop was designed for temperature indicator manufacturers and food retailers for the purpose of (1) developing rapid, quick and inexpensive temperature indicators and (2) disseminate the new USDA message to consumers of using a thermometer for cooking beef patties. Information from the ARS-FSIS premature brown study and ARS ancillary studies on temperature variability was provided at this workshop. As a result of this meeting, an association was formed (Food Temperature Indicator Association) to conduct research and perform consumer affairs regarding temperature devices. Also several major retailers (i.e., Giant) have initiated ground beef thermometer usage programs employing ARS-FSIS research findings on safe-cooking of beef patties.

PUBLICATIONS:

Berry, B.W. 1998. Cooked color in high pH beef patties as influenced by fat content and cooking from the frozen or thawed state. J. Food Sci. 63:797-800.

Berry, B.W. and M.E. Bigner-George. 1999. Properties of beef patties cooked to elevated internal temperatures as a means of reducing pink color. J. Muscle Foods. (in press)

Bigner-George, M.E. and B.W. Berry. 1999. Thawing prior to cooking affects sensory, shear force and cooking properties of beef patties. J. Food Sci. (in press)

Moeller, S., D. Wulf, D. Meeker, M. Ndife, N. Sundararajan and M. B. Solomon. 1999. Impact of the Hydrodyne process on tenderness, microbial load, and sensory characteristics of pork longissimus muscle. J. Anim. Sci. 77:2119-2123.

Sair, A.I., A.M. Booren, B.W. Berry and D.M. Smith. 1999. Residual triose phosphate isomerase activity and color measurements to determine adequate cooking of ground beef patties. J. Food Protect. 62:156-161.

PROCEEDINGS/ABSTRACTS:

Berry, B.W., D.L. Soderberg and M.E. Bigner-George. 1999. Thermographic profiles of cooked beef patties as influenced by internal temperature and patty state prior to cooking. IFT, Chicago, IL Abstracts p 182.

Berry, B.W. and M.E. Bigner-George. 1999. Color properties of ground beef patties cooked on an outdoor gas grill as influenced by fat content, product handling and internal temperature. Proc. 10th Cong Food Sci and Tech.

Solomon, M.B., Z. Holzer, A.M. Campbell, B.W. Berry and A.M. Spanier. 1999. Effect of hydrodynamic pressure and the koshering process alone or in combination on beef strip loins. Proc. 45th Int'l. Cong. Meat Sci. and Techn. Vol II, pp412-414.

Williams-Campbell, A., Z. Holzer and M.B. Solomon. 1999. Effect of hydrodynamic pressure and the koshering process alone or in combination on vacuum packaged beef strip loins. BARC Poster Day, #56.

POSTMORTEM MUSCLE/MEAT CHANGES THAT AFFECT PRODUCT SAFETY AND QUALITY

ARS Contact Persons:	CRIS NUMBER:	6612-41420-005
C.E. Lyon,	FSIS NUMBER:	R3C101-05
S.D. Senter, L.L. Young	CRIS TERM. DATE:	June 2000

Russell Research Center
Poultry Processing & Meat Quality Research Unit
Athens, Georgia
Phone: 706-546-3418
Fax: 706-546-3633
E-mail: glyon@ars.usda.gov

OBJECTIVE A: Evaluate hand-held instrumentation for use in testing packaged/stored poultry meat for spoilage.

PROGRESS A: Hand-held instruments designed by Perkin Elmer Photovac to quantitate volatile organic compounds were tested for use in determining the quality of stored chicken meat. The instruments, designed to measure total ionizable compounds and specific compounds in ppb quantities, did not provide data that correlated significantly with GC/MS or sensory analysis of headspace volatiles. The CRADA with PE Photovac was terminated by mutual consent of the two parties.

IMPACT/TECH TRANSFER A: Data showed that instruments used did not produce reliable indicators of quality. These data were presented to PE Photovac. Data relating to analysis of the volatile organic compounds formed during storage of the meats were reported to the literature for publication.

PUBLICATIONS:

Senter, S.D., L.L. Young, B.G. Lyon and C.E. Lyon. Evaluation of Animal Age and Muscle Type on Beef Log Juice Color and Residual GOT Activity when Cooked to Target End-point Temperature of 79.4°C. J. Sci Food & Agric. (in press)

RAPID PATHOGEN DIAGNOSTIC AND DETECTION METHODS

ARS Contact Persons:
J. B. Luchansky
G. Richards

CRIS NUMBER: 1935-42000-030
FSIS CODE:
CRIS TERM. DATE: September 2003

Delaware State University
Microbial Food Safety Research Unit
Dover, DE 19901
Phone: 302-857-6419
Fax: 302-739-5198
E-mail: grichard@dsc.edu

OBJECTIVE: Develop rapid detection and monitoring methods for pathogen and spoilage microorganisms in aquaculture processes and products and improve depuration efficiency to prevent human illness.

PROGRESS Renovations to the new worksite were completed in December 1998, the laboratories were equipped, a project statement in an area new to ARS was submitted, new personnel were hired and research was initiated. Studies were begun on the development of more rapid methods for the detection of infectious viruses and vibrio bacteria in shellfish, with emphasis on hepatitis A and Norwalk viruses using poliovirus as a model, and on *Vibrio vulnificus*. A collaborative study was completed that involved the National Ocean Service in Charleston, SC, the Virginia Polytechnic Institute and State University in Blacksburg, VA, and the ARS Dover worksite. The collaboration focused on the use of pulsed field gel electrophoresis to identify sources of *E. coli* contamination that can affect molluscan shellfish safety.

IMPACT/TECH TRANSFER: Five presentations were given at National and International meetings concerning virus contamination of shellfish. A symposium was also organized and chaired at the Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians on methods for detecting infectious viruses in foods.

PUBLICATIONS:

Richards, G.P. 1999. Limitations of molecular biological techniques for assessing the virological safety of foods. J. Food Protect. 62:691-697.

Richards, G.P. and D.O. Cliver. 1999. Foodborne Viruses. Compendium of Methods for the Microbiological Examination of Foods, Chapter 46, American Public Health Assoc., Washington, DC. (in press)

DETECTION AND TREATMENT OF LISTERIA AND OTHER BACTERIA IN CHANNEL CATFISH

ARS Contact Persons:
J.A. Lindsay, C. White Specific Co-operative Agreement 58-6202-5-083

Center of Food Safety and Post-Harvest Technology
Mississippi State University
Box 9805, Mississippi State, MS 39762
Phone: 601-325-3200
Fax: 601-325-8728
E-mail: Ruth@Ra.MsState.edu

OBJECTIVES: The major objective of this study is to ensure to the consuming public a safe and high quality catfish/and related foods product, work is being done to assess and rapidly detect any foodborne pathogens or inhibitory substances in the commodity. Commensurate with the development of primarily microbial detection techniques is the effort being exerted in optimizing processing and packaging operations to further enhance product safety. Novel processing procedures can possibly be utilized in the value-added segment of channel catfish to extend product shelf-life without sacrificing safety or quality. The development of new products from catfish has escalated. The distribution system is ever expanding. Both of these trends have caused a major emphasis on food safety and food quality (especially as related to expected and actual shelf-life). Due to the commercial success of the catfish industry, new catfish "farmers" have entered the market which increases food safety interest.

PROGRESS: There were several major accomplishments conducted this past year in the project. In most years, it is relatively easy to identify one accomplishment as being the most significant; however, this year there were two really important accomplishments. The first had to do with the continued understanding and removal of biofilms in the processing/packaging environment. These results can positively impact the catfish processing industry. The other significant accomplishment resulted from the use of high frequency ultrasound to eliminate pathogens in chiller/processing water.

IMPACT: Major accomplishments over the life of the project
The presence of foodborne pathogens, e.g., *Listeria monocytogenes*, has been verified. The effectiveness of various antimicrobials (EDTA, monolaurin, nisin, lactic acid, trisodium phosphate) has been evaluated on freshly processed channel catfish. The presence and most effective killing procedures were noted for *Edwardsiella tarda*, *L. monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* 0157:H7 and *Aeromonas hydrophila*.

Part III. Post Slaughter Pathogen Modeling and Control

Modified atmosphere packaging has been shown to increase shelf-life of the product, e.g., fillet, and, in combination with other processes (antimicrobials and irradiation), would minimize pathogen growth. Holding fish fillets at 2°C under normal or modified atmosphere did not affect *L. monocytogenes* growth. However, dipping in 0.5% acetic or 0.003% peroxyacetic acids appeared to inhibit this pathogen (at 2°C). Ozone and hydrogen peroxide at concentrations of 10 ppm and contact times from 3-10 (to 15) minutes helped extend microbial shelf-life but "bleached' the product. Also, catfish fillets were packed under MAP, Master Pack and air-pack and were inoculated with a *C. botulinum* cocktail and stored at 2 and 10°C. In all treatments, *C. botulinum* toxin formation followed or was parallel to microbial (> log 7 CFU/g) or sensory (odor) spoilage. The Master Pack system appeared to be the most effective in inhibiting toxin formation while increasing shelf-life.

The published solid phase extraction methodology (Long et al., J. Assoc. Off. Anal. Chem. 73:864-867, 1990) continued to be a suitable method for extraction of oxytetracycline from catfish muscle prior to analysis by HPLC. An experiment was conducted using high dosage levels (7.7 mg/100g body weight/day) administered to a range of fish sizes. Residues were highest on days 1 and 2 after cessation of treatment, and residues were substantially lower by days 4 and 7. The largest (73-92g) and smallest (26-28g) fish appeared to retain higher residues than the fish with intermediate weights.

The *in vivo* pathogenicity of *Listeria* was completed and hemolytic, lecithinase (phosphotidylcholine-phospholipase C, PC-PLC) and phosphotidylinositol-phospholipase (PI-PLC) activity were determined. A panel of monoclonal antibodies was produced and tested for activity to listeriolysin O (LLO) and PLC. All strains of *Listeria* tested were positive for LLO production with the exception of ATCC 15313.

An ELISA-mediated PCR technique was developed to detect and quantify *L. monocytogenes* in food products. The detection limit for *L. monocytogenes* experimentally inoculated into channel catfish fillets was 1-2 CFU/g. Little or no cross reaction was detected in the presence of other spoilage and pathogenic organisms.

Future work

Pathogen testing on fresh channel catfish will be continued. Specific pathogens to be tested are *S. typhimurium*, *L. monocytogenes* and *E. tarda*. Specific antimicrobials will be tested against these pathogens. An evaluation will be made of a novel steam pasteurization device developed by USDA-ARS-ERRC to kill surface microorganisms on fresh deheaded and eviscerated catfish.

EDTA, monolaurin and nisin will be assayed for antimicrobial activity against *E. coli* 0157:H7 using gradient plates. Also, various concentrations of common sanitizers will be prepared and assayed following AOAC methods. Use of impedance microbiology techniques should result in a rapid and accurate method of determining sanitizer strength.

Minimum growth temperature of *Streptococcus iniae* will be determined as well as a study of the anitmicrobial efficacy of monolaurin against this bacterium.

Use of processing aids and preservatives will be studied to evaluate their effectiveness versus pathogens in the product. The effect of handling stress and prechilling on fish muscle quality and shelf-life, and on safety of the product, will be studied. Use of processing aids or additives will be studied as a means of increasing shelf-life, and their effect on product safety evaluated.

A different batch of fish of varying sizes will be used to evaluate oxytetracycline residues. A new HPLC column will be used. This may eliminate the interference of some of the components in plasma samples (a more convenient analytical technique). Also, an investigation will be made into the possible metabolism of oxytetracycline.

Pathogenicity testing will continue with emphasis on neonatal and geriatric mice. In addition, there will be evaluation of channel catfish *L. monocytogenes* isolates for production of LLO and PLC activity using the LLO and PLC MAbs generated in the lab this past year.

Efforts will be made to continue to evaluate the accuracy of QT-PCR to detect uninjured and injured *L. monocytogenes* by modifying procedure and testing more samples. Also, attempts will be made to shorten the time required to perform the ELISA mediated PCR by improving the procedure.

PUBLICATIONS:

Bal'a, M.F., and D.L. Marshall. 1999. Food antimicrobial susceptibility testing. In: Food System Functionality - Physicochemical and Biological Aspects". Z.U. Haque (Ed.). Technomic Publishing Co., Inc., Lancaster, PA. (in press)

Bal'a, M.F., J.D. It and D.L. Marshall. 1999. Moderate heat or chlorine destroys *Aeromonas hydrophila* biofilms on stainless steel. Dairy, Food Environ. Sanitat. 19:29-34.

Bal'a, M.F., R. Podolak and D.L. Marshall. 1999. Steam treatment reduces skin microflora population on deheaded and eviscerated whole catfish. Food Microbiol. (in press)

Erdenlig, S., A.J. Ainsworth and F.W. Austin. 1999. Production of monoclonal antibodies to *Listeria monocytogenes* and their application to determine virulence of isolates from channel catfish. Appl. Environ. Microbiol. 65:2827-2832.

Kim, T.J. 1999. Biochemical, physical, microbiological, and sensory traits of catfish fillets treatedwith selective antimicrobials and sanitizers. M.S. Thesis, Dept. Food Science and Technology, Mississippi State University.

Marshall, D.L., L.N. Cotton and C.F. Fernandes. 1999. Chlorine usage in fish and seafood processing. In: Chlorine Usage Guidelines in Food Processing". Chlorine Chemistry Council. (in press)

Suvanich, V., D.L. Marshall and M.L. Jahncke. 1999. Microbiological and color quality changes of channel catfish frame mince during chilled and frozen storage. J. Food Sci. (in press)

Wang, C. and J.L. Silva. 1998. Prevalence and characteristics of *Aeromonas* species isolated from channel catfish. J. Food Protect. 62(1):30-34.

Wang, C. and C. Hong. 1999. A rapid PCR-based hybridization assay for the detection of *Listeria monocytogenes* in channel catfish. Food Microbiol. 16:291-297.

Wang, C. and C. Hong. 1999. Quantitative PCR for *Listeria monocytogenes* with colorimetric detection. J. Food Protect. 62:35-39.

PROCEEDINGS/ABSTRACTS:

Chamul, R.S., J.L. Silva and T.J. Kim. 1999. Optimization of pH, contact time, and mechanical shear of peroxyacetic acid solutions to treat channel catfish fillets. 60th Annual Meeting of IFT, Chicago. Abstr. No. 79E-6, pp. 229.

Kim, J. and D.L. Marshall. 1999. Trisodium phosphate inactivation of *Salmonella typhimurium* and *Listeria monocytogenes* attached to catfish skin. 60th Annual Meeting of IFT, Chicago. Abstr. No. 79C-20, pp. 217-218.

Nuñez, A.L., J.L. Silva and R.S. Chamul. 1999. Onset of rigor and quality of channel catfish (*Ictalurus punctatus*) fillets as affected by pre-processing handling and transport. 60th Annual Meeting of IFT, Chicago. Abst. No. 79E-32, pp. 234.

Nuñez, A., J.L. Silva and R.S. Chamul. 1999. Evaluacion de la influecia diferentes metodos de manejo y transporte antes de procesamiento sobre la variacion del grado de frescura (valor K) en filetes refrigerados de bagre de canal. II Congreso Venezoalno de Ciencia y Tecnologia de los Alimentos, Caracas, Venezuela.

Silva, J.L., T.J. Kim and R.S. Chamul. 1999. Influence of selected additives on microbial load and some pathogens of channel catfish fillets. 60th Annual Meeting of IFT, Chicago. Abst. No. 79E-4, pp. 229.

PART IV. RESIDUE DETECTION AND CHEMICAL ANALYSIS

ADVANCED TECHNOLOGIES FOR THE ANALYSIS OF CONTAMINANTS IN MEAT, POULTRY, EGGS AND OTHER FOOD PRODUCTS

ARS Contact Persons:
D.W. Thayer
W. Fiddler, S.J. Lehotay
J.W. Pensabene, M.J. Schneider

CRIS NUMBER: 1935-42000-027
FSIS CODE: E2CC03
CRIS TERM. DATE: September, 2000

Eastern Regional Research Center
Food Safety RU
Wyndmoor, PA 19038
Phone: 215-233-6502
Fax: 215-233-6642
E-mail: wfiddler@arserrc.gov

Dr. Lehotay moved from ARS in Beltsville to ERRC in July of 1999. Since he was working one-half of his time under CRIS number 1270-42000-003-00D, Development of Methods of Analysis for Residues in Meat and Other Agricultural Commodities, on FSIS-related work under their code number of B5CC01, it was decided to include the results of his work in this report (see objective E).

OBJECTIVE A: Develop supercritical fluid extraction (SFE) methods using carbon dioxide for the isolation of veterinary drug residues from meat tissues and eggs at or below the regulatory tolerance levels.

PROGRESS A: Several classes of drugs are used illegally for growth promotion purposes in animal production. Among these are the thyroid inhibitors, also called thyreostats. Based on our previous experience with chloramphenicol and sullen-amides and the desire to broaden the applicability of SFE technology, we thought these compounds would be amenable to isolation by SFE, even though the 2-thiouracil thyreostats are polar compounds. They include the parent, 2-thiouracil and its 5- and 6-methyl-, 6-propyl- and 6-phenyl- derivatives. At 10,000 psi and temperatures up to 150^{0}C with off-line SPE collection, supercritical CO_2 (SF-CO_2) alone failed to extract them from chicken muscle, used as a model tissue. SF-CO_2-modified with addition of 2.5-5.0% methanol as a cosolvent, with off-line trapping in methanol also failed. Even with substantial cleanup, the extracts were just too dirty. There was also no indication of peaks corresponding 6-propyl-2-thiouracil and its parent when analyzed by GC-NPD. Clearly, the thiouracils are either poorly soluble in modified or unmodified SF-CO_2 or significantly bind to sample components. To overcome this, attempts were made at *in-situ* derivatization with methyl iodide, which were also unsuccessful. Current investigations are being carried out using inverse SFE, where SFE is used as a cleanup technique to remove all the contaminants from the sample, then the contents of the extraction vessel eluted with solvent for the analytes in question.

Another class of drugs, beta-agonists (BAs), whose mechanism for growth promoting activity is different, was also studied by SFE. Earlier work by a Visiting Scientist to ERRC indicated that one member of this class, clenbuterol, the most commonly used and abused BA could be effectively isolated by SFE from bovine liver tissue. In this case, the clenbuterol was detected directly in the extract by enzyme immunoassay (EIA). These results suggested that other members of the same class of BAs might also be extractable by SFE. To develop a true multiresidue approach applicable to six or more BAs, a different means of analysis was required. We carried out studies on the use of methyl-boronic acid to form volatile boronic acid ester derivatives that had been successfully employed for BAs in bovine urine. This would make them amenable to separation and determination by GC-mass selective detection (MSD). First, we established the optimal conditions required for effective off-line derivatization of these BAs using clenbuterol as the model compound. Extracts with interference and variable recoveries were obtained using the same SFE conditions as previously used with off-line collection on acid-washed Celite. It was thought that derivatization in the extraction vessel, *in-situ*, would offer other advantages. Here again, interference with the detection of clenbuterol were encountered with the source finally traced to the polypropylene wool or polypropylene frits used to segment the sample in the extraction vessel. Glass wool fiber filters seem to overcome this problem and the work continues using this derivatization approach.

In the attempt to employ SFE to replace the more solvent intensive methods for drug and pesticide residues currently available, it is often difficult to predict whether certain compounds of intermediate or greater polarity can be solubilized in supercritical carbon dioxide or isolated in this manner. Solubility studies were carried out on several classes of drugs, including amphenicols, beta-agonists and hydroxybenzoic acids by a recirculating fluid extraction method to help derive predictive equations.

Trace levels of veterinary drug residues require SFE conditions that can vary widely in temperature and pressure. When these analytes are extracted from animal tissue, lipids are often extracted with them. These lipids may interfere with their chromatographic analysis and, at some point, need to be separated. Neutral alumina, a common sorbent used for this purpose, was evaluated as an in-line trap for lipids from chicken liver using a pressure range of 490-680 bar and temperatures of 40 and 80^{0}C. At 80^{0}C and 680 bar, <1.5% of the extracted total lipids were trapped in-line. At 40^{0}C and 490 and 680 bar, 30 and 18%, respectively, were trapped in-line. As determined by an HPLC-Evaporative Light Scattering Detector (ELSD), of the different classes of lipids, mostly fatty acids and cholesterol were trapped. Under these SFE conditions, alumina would not be a good in-line sorbent for lipid separation.

IMPACT/TECH TRANSFER A: Obtaining information on residues from these two prohibited classes of drugs in animal-derived foods, which can cause adverse effects in humans is essential. Current laboratory methods, where available, have significant limitations and are generally solvent intensive. The application of a solvent-sparing technique like SFE for drug residue analysis has a number of advantages since they combine several steps in the analytical procedure. The use of *in-situ* derivatization would add additional flexibility to the technique. SFE is a powerful technique that is

under-exploited for residue analysis. Screening methods for residues of these potentially toxic compounds will allow the regulatory agencies to more effectively monitor/screen for them. The research on SFE extraction conditions was performed to help obtain a better understanding of the SFE process that will aid in future work and perhaps increase productivity by reducing the amount of time it takes for exploratory investigations of a particular class or individual compounds

OBJECTIVE B: Investigate the use of automated on-line micro-dialysis as a sample preparation (analyte extraction and concentration) technique for antibiotic residues in foods.

PROGRESS B: Previous work has focused on the application of this aqueous-based automated microdialysis (ASTED)-HPLC method for the isolation, cleanup and determination of fluoroquinolone (FQ) antibiotics, primarily of oxolinic acid, flumequine and sarafloxacin in chicken liver tissue at levels as low as 5 ppb. Only the latter compound is a true FQ; the others are quinolone antibiotics that are used primarily in aquaculture and have very different physical properties. This microdialysis technique, in combination with an integrated HPLC and fluorescence detector system, has been successfully applied for the simultaneous analysis of multiple FQ residues in chicken eggs that include norfloxacin, ciprofloxacin, danofloxacin, enrofloxacin and desethylene ciprofloxacin in addition to sarafloxacin. Recovery studies on the six individual FQs were carried out on spiked eggs over a range of 0.2 to 200 ppb. Generally, the recoveries were >65% with RSDs <10%. The limits of quantitation varied from 0.3 to 3.0 ppb, depending on the specific FQ. Incurred enrofloxacin and sarafloxacin in eggs from dosed laying hens were successfully analyzed by this method. The results show that this is a viable and efficient automated method for analyzing FQs in eggs. Collaborative studies using this technique were carried out with FDA's CVM on enrofloxacin, ciprofloxacin, sarafloxacin and difloxacin in fortified and incurred bovine milk samples.

IMPACT/TECH TRANSFER B: The use of FQs in animal production is controversial because of increased concerns about human infections that are becoming more resistant to antibiotics currently in use. As such, it is important to determine whether this class of antibiotics is being misused or used illegally. At present, there is very little information on the presence of FQ residues in edible meat, poultry tissue and eggs. This information is needed since their presence may contribute to antibiotic resistance. The technique reported offers several advantages that include automation, ppb sensitivity, clean extracts and good precision with minimal use of organic solvents, all of which contribute to significant cost-efficiency (more samples analyzed per day). To date, two FQ antibiotics have been approved for use in animal production, but others are pending approval. This technique is currently being used in FDA-CVM laboratories and is likely to be used in further studies on FQ metabolism and pharmacokinetics.

OBJECTIVE C: Apply SFE technology to the isolation of polychlorinated dibenzo-p-dioxins (PCDDs, dioxins) from animal-derived products.

PROGRESS C: We have previously reported on the difficulties in applying SFE for isolating PCDDs from eggs at the low ppt level and obtaining extracts that were sufficiently clean for GC-electron capture detector (ECD) analysis, without the use of GC-High Resolution Mass Spectrometry (HRMS). Then the switch in emphasis was made to ball clay that was used as an anti-caking agent in animal and fish feed that ultimately caused it to be banned from use for this purpose. Ball clay samples were obtained from both the EPA and from a local supplier of ball clay used for making ceramic objects. The optimal conditions required for extracting PCDDs were 10,000 psi and a temperature of 150^{0}C. GC-ECD chromatograms were obtained that contained a number of apparent PCDDs which included very small peaks in the 2,3,7,8-tetrachloro-DD (TCDD) area that were not above background levels, and much larger peaks in the hepta- and octachloro-DD (HpCDD, OCDD) area. The congener patterns from each of the clay SFE extracts from the various mines were different. More noteworthy, was a very large peak that appeared at a lower retention time than the PCDDs. This peak was later confirmed in one clay sample (TN5) as pentachlorophenol (PCP) by GC-MS/MS. Fairly large amounts (higher ppt) of OCDD and HpCDD and smaller amounts of HxCDD, and PtCDD were also confirmed. Another sample (TN10) contained larger amounts of OCDD and varying amounts of the other PCDDs, but no PCP. Another clay, (KYOM4), had the same homologues/congeners in varying amounts and no PCP. No polychlorinated dibenzofurans (PCDFs) were detected in any of the samples tested. One sample also contained PCBs. Because of the large amount of PCP in the TN5 ball clay sample, we questioned whether this might be the source of OCDD. Experiments were carried out to determine whether OCDD could be artifactually formed from PCP under the SFE conditions employed; it was. The absence of PCDFs suggested that combustion was not the source of PCDD formation. If it were of biogenic origin, all of the samples would be expected to contain PCP. Since ball clay goes through some treatments after mining, it is possible that it is contaminated at this point. The how and why PCDDs are present in the clay are still unknown.

IMPACT/TECH TRANSFER C: The recent episode in Belgium of dioxins (PCDDs) in animal-derived foods resulting from feed contaminated with PCDD-containing animal fat and the earlier one in the U.S. where PCDDs were found in chickens, eggs and catfish show the need for accurate, cost-effective, specific laboratory screening procedures. The availability of good reliable methods will lead to rapid adoption by the regulatory agencies. Results from our studies indicate that the analysis of these compounds, at the ppt level, is extremely difficult because of interfering contaminants. Another factor is that artifactual formation of PCDDs can occur, especially when polychlorinated phenols are also present in the sample. SFE is widely used for PCDD analysis in environmental samples where these phenols may be present. To date, the application of SFE to foods for residue analysis has been limited, but there is a role for this technique. Our current work will help define the limitations of its use and opportunities for its effective application.

OBJECTIVE D: Apply SFE technology for the isolation of triazine (TRZ) herbicides from eggs.

PROGRESS D: Last year we reported the successful isolation by SFE of ten triazines, members of an important class of pre-emergent weed control herbicides that are commonly used on animal food

crops, from fortified eggs with recoveries > 70% for levels as low as 100 ppb. Often the extractability of analytes is different in spike-recovery studies than with those containing normally incurred analytes. For this reason, we analyzed eggs from laying hens fed atrazine, the most commonly used triazine herbicide. It was apparent that very little of the parent triazine is translocated into the eggs; however, two of the desalkyl metabolites were present in higher concentrations. The findings indicate that methods suitable for the analysis of triazines should also include their principal metabolites. The SFE results were found to be superior to those obtained by a solvent extraction published in the FSIS methods manual. This new method has an analysis time of 1.5 hours and uses only 8 ml of non-halogenated solvents and exhibited excellent performance characteristics with clean chromatograms obtained by GC-NPD.

IMPACT/TECH TRANSFER D: Under current risk assessment guidelines it is expected that triazine herbicides will continue to be used, but additional information is needed on triazine residues in foods for the planned reassessment by EPA. It is especially important that animal-derived products, including eggs, be monitored to obtain this kind of data. The extensive use of these herbicides on animal feed crops, especially corn, is highly controversial since several members of this class exhibit strong immunotoxicity and carcinogenic activities.

OBJECTIVE E: Develop quantitative multiresidue methods of analysis for pesticide residues in meat and other agricultural products that are suitable for regulatory purposes, emphasizing the use of gas chromatography (GC), HPLC, mass spectrometry (MS), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), capillary electrophoresis (CE), and other modern techniques.

PROGRESS E: Direct Sample Injection/Gas Chromatography/Tandem Mass Spectrometry (DSI/GC/MS-MS). A DSI/GC/MS-MS method of analysis was developed and evaluated for nonfatty (fruits and vegetables) and fatty foods (eggs and meats). The DSI approach is rapid, easy, inexpensive, rugged, and permits large volume injection of dirty extracts. The method entails extraction of the sample with acetonitrile in a homogenizer followed by salting out of water and a 5 min. centrifugation step. Up to 30 μL of the dirty extract is transferred to a disposable vial that is placed in the GC inlet. During injection, the volatile solvent is evaporated and vented, and the semi-volatile analytes are introduced onto the GC column. Since the nonvolatile components remain in the vial, they do not contaminate the GC system. The capillary GC column cryofocuses the semi-volatile components then separates the analytes for subsequent MS-MS detection. The use of the universally selective MS-MS detection technique permits simultaneous analysis and confirmation of a wide range of targeted pesticides at ultratrace levels in the complex extracts.

In a study of DSI/GC/MS-MS of nonfatty foods, a mixture of carrots, apples, and green beans were made to make a more complex extract than the individual commodities would have provided. Furthermore, extracts containing incurred pesticides were diluted 3-fold to provide a more stringent test of this approach. In it, a comparison was made of DSI/GC/ MS-MS with DSI/GC/MS and the more traditional splitless liquid injection in GC/MS and GC/MS-MS modes. The results obtained from the DSI/GC/MS-MS approach were more accurate and precise, and unlike the other

approaches, this one required little analyst intervention during integration of the peaks. Furthermore, fat and meat extracts prepared by the FSIS CHC3 method were also analyzed by DSI/GC/MS-MS which provided detection limits below tolerance levels for selected organochlorine (OCPs), organophosphorus (OPPs), and pyrethroid pesticides and triazine herbicides with a single injection while achieving simultaneous confirmation and quantitation. Fatty foods that do not undergo a cleanup step can be screened for pesticides at low levels, but quantitation is not as precise.

SFE of Pesticides in Nonfatty Foods. A collaborative study, Determination of Pesticide Residues in Nonfatty Foods by SFE and GC/MS, was completed through the AOAC® Official Methods Program. Collaborators included 17 laboratories from 7 countries. Commodities included apple, green bean, and carrot which contained a total of 23 pesticides, representative of six classes (spiked, incurred, and for quality control). The pesticides were spiked in duplicate at 3 levels plus a blank in each commodity. Incurred and quality control pesticides were analyzed by a method of standard additions. At this time, the results have not been statistically evaluated, but the method is expected to meet acceptance criteria for most of the pesticides tested.

In a separate study involving SFE and GC/MS, the method was included in a European Union (EU) study comparing different methods of analysis for pesticide residues in nonfatty foods. Specially prepared samples of strawberry, spinach, carrot, apple, tomato, and wheat were provided by the EU and analyzed by the SFE and GC/MS method in the ARS lab. Other laboratories analyzed the incurred and spiked samples using traditional methods. At this time, the overall results have not been reported.

GC-Pulsed Flame Photometric Detection (PFPD). PFPD was evaluated for the detection of OPPs in pork fat and ground meat. The extraction procedure was the same as the FSIS CHC3 method used for the analysis of OCPs in fat. The results of our experiments verified that the OPPs (and other classes of pesticides) are also present in the same collection fraction as the OCPs obtained by the FSIS CHC3 procedure. The analysis of OPPs in the extracts using GC/PFPD was found to be reproducible and provide exceptionally low limits of detection. Currently, FSIS only uses GC-electron capture detection (ECD) in this analysis for the halogenated pesticides that does not detect nonhalogenated pesticides, such as many OPPs. The EPA has requested that FSIS also analyze OPPs to help meet goals outlined in the Food Quality Protection Act. The results of this project indicate that FSIS may simply use the PFPD with their existing method to expand the range of analytes tested in their monitoring program employing the same sample extract, without the need for additional extraction techniques.

IMPACT/TECH TRANSFER E: These studies impact the capabilities of regulatory and other laboratories that analyze pesticide residues in food. The use of lower cost, more rapid, easier, and higher quality methods can increase the monitoring rate, better ensure the absence of residues, and provide more accurate data for risk assessment purposes. Also, more reliable data will lead to better science-based regulations and policies. These recent findings had an even more direct effect on impact and tech transfer factors. More specifically, purchase of a PFPD was initiated by the FSIS

Western Lab for the analysis of OPPs on the basis of PFPD results described above. The ARS initiated AOAC International collaborative study on the analysis of pesticide residues in nonfatty foods led to the use of SFE and GC/MS by 17 laboratories in 7 countries. The application of this DSI/GC/MS-MS approach has spread to pesticide testing laboratories that have the instrumental capabilities. At least two state labs, in Montana and Nevada, have stated their desire to install the DSI apparatus on their present GC/MS-MS instruments. A United States - Israel Binational Agricultural Research and Development grant on the fast Analysis of Pesticide Residues in Agricultural Products was awarded to help support the DSI/GC/MS-MS and PFPD work.

PUBLICATIONS:

Cohen, E., R.J. Maxwell and D.J. Donoghue. 1999. Automated multi-residue isolation of fluoroquinolone antimicrobials from fortified and incurred chicken liver using on-line microdialysis and high performance liquid chromatography with programmable fluorescence detection. J. Chromatog. B, 724:137-145.

Fiddler, W., J.W. Pensabene, R.A. Gates and D.J. Donoghue. 1999. Supercritical fluid extraction of organochlorine pesticides from eggs. J. Agr. Food Chem. 47:206-211.

Hampson, J.W., R.J. Maxwell, S. Li, and R.J. Shadwell. 1999. The solubility of three veterinary sulfonamides in supercritical carbon dioxide by a recirculating equilibrium method. J. Chem. Eng. Data. (in press)

Lehotay, S.J. 1999. Analysis of pesticide residues in fruits and vegetable extracts by Direct Sample Introduction/Gas Chromatography/Tandem Mass Spectrometry (DSI/GC/MS-MS) J. AOAC Int'l. (in press)

Lehotay, S.J. 1998. New Technologies Applied in Pesticide Residue Analysis. In: Pesticide Residues '96: IV International Seminar on Pesticide Residues. A. Valverde (Ed.), Institute of Food Studies, Almería, Spain, pp. 53-76.

Lehotay, S.J., J.A. Harman-Fetcho and L.L. McConnell. 1998. Agricultural Pesticide Residues in Oysters and Water from Two Chesapeake Bay Tributaries. Mar. Poll. Bull. 37:32-44.

Lehotay, S.J. 1999. Strategies for Multiclass, Multiresidue Analysis of Pesticides. Encyclopedia of Analytical Chemistry, John Wiley & Sons, New York. (in press)

Lehotay, S.J. and F.J. Schenck. 1999. Multiresidue Methods (Extraction). Encyclopedia of Separation Science, I.A. Wilson, T.R. Adlard, C.F. Poole, M. Cook (Eds.), Academic Press, London. (in press)

Li, S., R.J. Maxwell and A.R. Lightfield. 1999. In-line and off-line trapping of lipids extracted from chicken liver by supercritical fluid extraction. J. Am. Oil Chem. Soc. 6:523-528.

Maxwell, R.J. and A.R. Lightfield. 1999. An improved method for efficient pre-development washing of HPTLC plates. J. Planar Chromatogr. 12:109-113.

Maxwell, R.J. and A.R. Lightfield. 1998. Multiresidue Supercritical fluid extraction method for the recovery at low ppb levels of three sulfonamides from fortified chicken liver. J. Chromatogr. A. 715:431-435.

Maxwell, R.J., E. Cohen and D.J. Donoghue. 1999. Determination of sarafloxacin residues in fortified and incurred eggs using on-line microdialysis and HPLC/ fluorescence detection. J. Agr. Food Chem. 47:1563-1567.

O'Keeffe, M.J., M. O'Keeffe, J.D. Glennon, A.R. Lightfield and R.J. Maxwell. 1999.Supercritical fluid extraction of clenbuterol from bovine liver tissue. Analyst 123:2711-2714.

Pensabene, J.W., W. Fiddler and D.J. Donoghue. 1999. Supercritical fluid extraction of atrazine and other triazine herbicides from whole eggs. J. Agr. Food Chem. (in press)

Pensabene, J.W., W. Fiddler and D.J. Donoghue. 1999. The isolation of chlor-amphenicol from whole eggs by super-critical fluid extraction with in-line collection. J. AOAC Int'l. (in press)

Schenck, F.J. and S.J. Lehotay. 1999. Does further clean-up reduce the matrix enhancement effect in GC analysis of pesticide residues in food? J. Chromatog. A. (in press)

Stolker, A.A.M., L.A. Van Ginkel, R.W. Stephany, R.J. Maxwell, O.W. Parks and A.R. Lightfield. 1999. Supercritical fluid extraction of methyltestosterone, nortestosterone and testosterone at low ppb levels from fortified bovine urine. J. Chromatog. B. 726:121-131.

ABSTRACTS:

Fiddler, W. and J.W. Pensabene. 1999. Isolation of triazines from eggs by SFE with unmodified supercritical carbon dioxide. 50th Pittsburgh Conf., Orlando, FL. 2094P.

Fiddler, W. 1999. Application of supercritical fluid extraction to pesticide and drug residue analysis in eggs. 218th Am. Chem. Soc. Nat'l Meeting, New Orleans, LA. AGRO-3

Lehotay, S.J. 1999. Evaluation of Direct Sample Injection for GC/MS-MS analysis of pesticide residues. 50th Pittsburgh Conf., Orlando, FL. #1004.

Lehotay, S.J. 1999. Collaborative Study for the determination of pesticide residues in nonfatty foods by SFE and GC/MS. 11 th California Pesticide Residue Workshop, Sacramento, CA. #009.

Lehotay, S.J. and J.A. Harman-Fetcho. 1999. Direct Sample Introduction (DSI)/GC/MS-MS for the analysis of pesticide residues in eggs. 218 th Am. Chem. Soc. Nat'l Meeting, New Orleans, LA, AGRO-7.

Lehotay, S.J. 1999. Collaborative study to determine pesticide residues in nonfatty foods by SFE and GC/MS. 113 th AOAC Int'l Annual Meeting, Houston, TX, F-1202

Pensabene, J.W., W. Fiddler, J.M. Foster and D.J. Donoghue. 1999. A method for the supercritical fluid extraction of atrazine and other triazine herbicides from whole eggs. Inst. Food Technol. Annual Meeting, Chicago, IL. 50-C3.

Pensabene, J.W., W. Fiddler and D.J. Donoghue. 1998. The isolation of chloramphenicol from whole eggs by supercritical fluid extraction with in-line collection. 8th Int'l Symp. Supercritical Fluid Chromatography and Extraction. St. Louis, MO. C-06.

Schenck, F.J. and S.J. Lehotay. 1998. Does further cleanup reduce the matrix enhance ment effect in GC analysis of pesticide residues? FDA Science Forum, Washington, DC. #B7.

Schneider, M.J. 1999. A multiresidue method for the analysis of fluoroquinolones from eggs. 113th AOAC Int'l Annual Meeting. Houston, TX. #H-1401.

METHODOLOGY DEVELOPMENT FOR RAPID ANALYSIS OF DRUG AND PESTICIDE RESIDUES IN FOOD ANIMAL PRODUCTS

ARS Contact Persons: **L.H. Stanker, R.C. Beier, C.K. Holtzapple**

CRIS Number: 6202-42000-009
FSIS Code: C1CC03
CRIS Completion Date: March 2000

Food and Feed Safety Research Unit
Food Animal Protection Research Laboratory
College Station, TX
Phone: 409-260-9484
Fax: 409-260-9332
E-mail: holtzapple@ffsru.tamu.edu

OBJECTIVE A: To develop, evaluate, and provide confirmatory testing of highly sensitive, inexpensive, monoclonal antibody-based, immunoassays useful for detection and quantification of chemical residues in animal products and body fluids either on-the-farm, in-the processing plant, or for laboratory based analyses.

PROGRESS A: Development of new, monoclonal antibody based immunoassays are in progress for a number of compounds including: fluoroquinolones,- 4,4'dinitrocarbanilide, tylosin and tilmycosin.

Fluoroquinolones: Fluoroquinolones are antibiotics used to control *E. coli* in poultry. A series of monoclonal antibodies using sarafloxacin as the immunogen have been developed and characterized. These monoclonal antibodies also bind related fluoroquinolones. For example, these antibodies bind sarafloxacin, difloxacin, enrofloxacin, norfloxacin, and ciprofloxacin . These antibodies have been extensively characterized and one chosen for additional studies. This antibody has been used to develop a simple immunoassay for residue detection in chicken tissue and in eggs. A novel, on-line immunoassay format has been developed that incorporates both high-performance immunoaffinity chromatography (HPIAC) and traditional high-pressure liquid chromatography (HPLC). The HPIAC-HPLC assay is also capable of detecting up to six different fluoroquinolones in a single sample (see objective C for additional information).

IMPACT/TECH TRANSFER A: Rapid tests such as those developed here should help producers, as well as government agencies, to screen poultry products for the presence of fluoroquinolone residues. In addition, manuscripts describing the antibodies and methods have been published in the scientific literature.

4,4'-Dinitrocarbanilide: Nicarbazin is a drug comprised of a 1 to 1 molar ratio of 4,4'-dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol that is used to prevent outbreaks of cecal and intestinal coccidiosis in chickens. DNC is the active agent in nicarbazin and it is used by the

FDA to determine the nicarbazin residues in chicken tissues. Likewise, nicrabazin determination using the established FSIS method measures the levels of DNC. Due to the difficulty of using derivatives of DNC to produce antibodies, we have synthesized a number of mimics of DNC for use as immunogens. These studies have employed sophisticated computer assisted molecular modeling methods to aid in evaluating the potential of these mimics . A number of cell fusion experiments have been completed and preliminary screening experiments indicate that at least two separate anti-DNC monoclonal antibodies have been isolated that are capable of binding parent DNC in a competition enzyme linked immunosorbent assay. These studies have been initiated with a CRADA partner (International Diagnostic System Corp.).

Upon successful generation of suitable monoclonal antibodies, they will be incorporated into a simple immunoassay by our CRADA partner and be available as a kit for rapid diagnostics of this drug. In addition, a manuscript describing the computer assisted molecular modeling of these compounds has been published.

Tylosin and Tilmycosin : Both tylosin and tilmycosin are antibiotics. We have initiated experiments to produce sensitive immunoassays for both of these compounds. Good analytical methods that are rapid and definative for both of these compounds are generally lacking. We have completed a preliminary study of a synthetic process to produce suitable protein conjugates of these molecules for use in producing monoclonal antibodies.

These studies have been initiated in association with a major pharmaceutical company as a CRADA partner. Upon completion, these assays will be incorporated into screening tests to assist in the application of these compounds and in residue detection schems.

Other Compounds and Activities: We continue a collaboration with Dr. Bruce Hammock, University of California-Davis, in studies to produce anti-dioxin immunoassays and analogs for dioxin. These studies utilized low-energy molecular models of the polychlorinated dioxins and furans and novel synthetic approaches to production of dioxin-protein conjugates to serve as immunogens. Likewise, we continue our collaborative studies with ARS scientists aimed at developing immunoaffinity chromatography methods for analysis of dioxin using a monoclonal anti-dioxin antibody generated earlier. A long standing collaboration with Dr. Steve Safe, Texas A&M University dealing with the function of the dioxin receptor continues.

Development of rapid tests for analysis of dioxins in foods and biological fluids would be highly desirable since present technology is time consuming and costly (in excess of $1500/sample). A U.S. patent was issued describing the development of monoclonal antibodies to the antibiotic drug ceftiofur.

OBJECTIVE B: To produce highly specific monoclonal antibody-based immunoassays capable of detecting enteric pathogens and normal gastrointestinal bacteria in poultry and swine.

PROGRESS B: Antibody reagents have been extensively used in biology and medicine to detect and confirm the presence of pathogens. The high level of specificity, speed, and simplicity of immunoassays as well as their low cost make these assays attractive for large scale screening or in clinical settings where rapid confirmation is necessary. A series of monoclonal antibodies to normal swine and poultry intestinal bacteria have been produced and used to develop rapid assays. These assays have been used to enumerate bacterial levels in both defined competitive exclusion (CE) cultures developed by the Unit and to track the fate of these bacteria in normal versus CE treated animals. In addition, monoclonal antibodies have been produced to pathogenic enteric bacteria including *E. coli* O157:H7, *Salmonella typhimurium* (phase II i antigen), and *Champylobacter* species. The anti-Campylobacter monoclonal antibodies fall into 4 groups: anti-*C. jejuni* specific antibodies; anti-*C coli* antibodies; anti-*C. lari* antibodies and a fourth group of monoclonals that bind equally to these three species. Monoclonal antibodies have also been generated that bind primary swine isolates of *Arcobacter*, and *Helicobacter* . Using a novel immunoassay format that incorporates an immunomagnetic electrochemiluminescence sensor we have demonstrated that we can detect as few as 1000 bacteria.

IMPACT/TECH TRANSFER B: The results of our studies enumerating the normal gut bacteria will provide useful information on the mechanisms controlling the effectiveness of competitive exclusion as an intervention strategy for control of pathogens on the farm. Such information will be essential for development of "next-generation" competitive exclusion cultures for pathogen control. The anti-Campylobacter antibodies potentially will form a rapid immunochemical method for species typing and confirmation. Clearly, development of a real-time immunoassay for a pathogen would have direct applications to the food animal industry.

OBJECTIVE C: The development of multianalyte immunoassays using an on-line, high-performance immunoaffinity chromatography column coupled with a traditional HPLC analysis.

PROGRESS C: Traditionally, immunoassay methods for residue analysis are viewed as single analyte methods. In reality, most antibodies, both polyclonal and monoclonal, have some degree of cross reactivity with structurally related drugs and even with some metabolites of the parent drug. In fact, cross-reactivity often is cited as a limitation of immunoassays requiring that results be expressed as drug equivalents versus drug concentration because of the uncertainty of which cross-reacting analyte is responsible for the signal. However, in principle, this inherent cross-reactivity of antibodies, particularly that of a well characterized monoclonal antibody, can be exploited to detect a number of related drugs with a single antibody. Using our anti-fluoroquinolone monoclonal we have demonstrated the ability to generate a multidimensional, multianalyte immunoassay. The assay is performed on an Intergral Microanalytical Workstation (Perkin Elmer Inc.) equipped with the ability to utilize multiple binding and separation columns, multiple reagents, and fluorescent detection in an automated mode under computer control. Briefly, sample extracts and/or mixtures of standards are first trapped on an in-line immunoaffinity column. This column is washed and bound material automatically eluted to a reverse phase column for analyte separation. We have quantitatively detected up to six fluoroquinolone antibiotics (difloxacin, sarafloxacin,

trovafloxacinin, enrofloxacin, norfloxacin, and ciprofloxacin) in a mixed standard fluoroquinolone library. We have extended these studys to analysis of multiple fluoroquinolones in complex biological matrixes including milk, tissue and serum. We also demonstrated that the fluoroquinolones could be differentially eluted as two groups (high and low affinity binders) from the immunocapture column. Experiments are underway to couple the immunocapture column with an electrospray mass spectrometer for on-line spectral confirmation of the residues detectd. The assay, as currently configured, is completed in less than 20 minutes and the instrument is capable of automatically injecting up to 105 samples.

IMPACT/TECH TRANSFER C: The ability to convert single analyte immunoassays to automated multianalyte immunoassays will greatly enhance the application of these methods for residue analysis. In addition, these studies are the first to demonstrate the ability to utilize the inherent differential affinity of antibody molecules to separate analytes based on their interaction with a single immunoaffinity column. Refinement of these methods may allow development of even more simplified methods using only a single immunoaffinity column to resolve complex mixtures of compounds. These studies have been published in the scientific literature.

OBJECTIVE D: To develop automated, immunochemical detection assays for residues and enteric pathogens using an Immunomagnetic Elecrochemiluminescence Biosensor.

PROGRESS D: Studies recently have been initiated to improve automation, and to reduce the number of steps often associated with traditional immunoassays such as the ELISA. We are employing an immunomagnetic electrochemiluminescence assay running on a commercial biosensor designed as a flow-through system. Use of more sophisticated detectors, such as luminescence, and automated instrumental methods, makes it possible to improve the sensitivity and reproducibility of immunoassays. Improving assay sensitivity will translate into more simplified sample preparation for residue analyses, and increase confidence in assays for detection and identification of bacteria. These studies are being extended to the detection of *Campylobacter* and *Salmonella* bacteria with the goals of improving the detection limits and development of a detection scheme that can detect microbes in real-time with no, or only very limited, tissue culturing requirements. Using a simple sandwich format we have been able to detect 1000 bacterial particles in a sample. Our aim is to reduce this by an order of magnitude. Finally, using the anti-ceftiofur monoclonals described above, we have demonstrated the application of this instrumental method for detection of residues.

IMPACT/TECH TRANSFER D: The development of sensitive instrumental immunoassays will greatly facilitate the transfer of immunochemical methods into analytical and bacterial laboratories. The application of multipurpose instruments using formats where only the antibody needs to be supplied greatly reduces the reliance on multicomponent immunoassay kits. Such assay formats should reduce the time between antibody development and assay availability. We are exploring the development of a CRADA with the manufacturer of this immunomagnetic electrochemi-luminescence.

PUBLICATIONS:

Beier, R.C., C.R. Young and L.H. Stanker. 1998. Detection of bacteria from a cecal anaerobic competitive exclusion culture with an immunoassay electrochemiluminescence sensor. pp. 10–20. *In* "Pathogen Detection and Remediation for Safe Eating." H. Early, M.B. Medina, A.G. Rand, A.G. Senecal, S.-I. Fu and T.C.S. Yang (eds). The International Society for Optical Engineering (SPIE) Vol. 3544, Boston, MA, pp. 168

Muldoon, M.T., I.A. Font, R.C. Beier, C.K. Holtzapple, C.R. Young and L.H. Stanker. 1999. Development of a cross-reactive monoclonal antibody to sulfonamide antibiotics: evidence for structural conformation-selective hapten recognition. Food Agric. Immunol. 11(2):117–134.

Holtzapple, C. K. and L.H. Stanker. 1998. Affinity selection of compounds in a fluoroquinolone chemical library by on-line immunoaffinity deletion coupled to column HPLC. Anal. Chem. 70:4817-4821.

Holtzapple,C. K., R.J. Carlin and L.H. Stanker. 1998. Incorporation of molecular modeling techniques in immunogen design and immunoassay development. Rec. Res. Devel. Agric. Food Chem. 2:865-880.

Holtzapple, C. K., S.A. Buckley and L.H. Stanker. 1999. Determination of four fluoroquinolones in milk by on-line immunoaffinity capture coupled with reversed-phase liquid chromatography. J. Assoc. Org. Anal. Chem. Int. 82:607–613.

Holtzapple, C. K., S.A. Buckley and L.H. Stanker. 1999. Immunosorbents coupled on-line with liquid chromatography for the determination of fluoroquinolones in chicken liver. J. Agric. Food Chem. 47:2963–2968.

PATENTS:

Stanker, L. H., B. Rose, C.K. Holtzapple and J. DeLoach. 1999. Monoclonal antibodies to ceftiofur. U.S. Patent Number 5,908,781.

DIOXINS AND OTHER ENVIRONMENTAL CONTAMINANTS IN FOOD

ARS Contact Persons:
J.K. Huwe, G.L. Larsen,
H. Hakk, W.L. Shelver

CRIS NUMBER: 5442-42000-002
FSIS CODE: F1CC01,02
CRIS TERM. DATE: September, 2001

Biosciences Research Laboratory
Red River Valley Agricultural Research Center
Fargo, ND
Phone: 701-239-1288
Fax: 701-239-1430
E-mail: huwej@fargo.ars.usda.gov

OBJECTIVE A: Identify residues of chlorinated dioxins and other environmental contaminants in animals raised for food and develop effective remediation procedures for these animals and their environments in order to minimize economic and consumer health consequences.

PROGRESS A: During a national-wide survey of dioxins in beef cattle, we observed that, although most levels were low, elevated concentrations of dioxins in beef could invariably be traced to pentachlorophenol (PCP) treated wood in feeding facilities. In order to determine the most effective remediation for PCP treated wood, depth profiles of dioxins in treated wooden posts were examined. The results showed dioxins penetrating to the center of the posts. A surface treatment with bleach was also investigated for remediation but was not adequate to destroy all dioxins in the wood. Based on the depth of penetration of the PCP and its impurities, more effective remediation methods may include sealing in the contaminants with paint or discouraging the use or reuse of treated wood on farms where animals are raised for food.

Since PCP appears to be a major source of dioxin residues in beef, we investigated whether PCP levels in animal tissues could be used as an indicator of dioxin contamination. PCP analyses require less rigorous cleanup procedures than dioxin analyses, are less expensive, and therefore may be more practical for widespread monitoring. We developed methods for analyzing PCP in beef adipose tissue and applied this methodology to twenty samples of beef collected from various sites across the U.S. The results showed that unless animals were held in a confined area with a constant exposure to PCP treated wood, the PCP levels in tissue did not adequately reflect the dioxin body burden. Because of the rapid excretion and metabolism of PCP (half life < 2 d) compared to that of dioxins (half life >2 months) it is not surprising the levels in tissue do not correlate. One parameter which may prove indicative of PCP exposure as the source of dioxins is the percent of the total dioxin equivalency (TEQ) due to a single congener, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin. This congener accounted for 40 - 47% of the TEQ in animals with known PCP exposures. Dr. George Fries, ARS, Beltsville, MD, has also shown that 1,2,3,6,7,8-HxCDD correlated with total dioxin levels in treated wood from feeding facilities in other locations.

One important factor in the toxicity of dioxins is their resistance to metabolism in mammalian systems. Metabolism studies conducted in this laboratory and others have shown that metabolism increases excretion of dioxins and may decrease toxicity. Mechanisms which enhance metabolism are a means of reducing body burdens. A metabolism study of the toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) was conducted in male rats which had been pretreated with either a nontoxic dioxin or β-naphthoflavone to induce microsomal enzyme systems. Rats were dosed with ^{14}C-labeled 2,3,7,8-TCDD after preinduction, and the extent of metabolism was followed by assaying the bile for radioactivity. The results showed that one nontoxic dioxin, 1,3,7,8-TCDD, increased the ability of the rats to metabolize 2,3,7,8-TCDD. The other treatment groups showed no difference compared to controls. Further studies are planned to investigate which enzyme systems were responsible for the enhanced metabolism and excretion and whether this may prove to be an effective remediation method for contaminated animals.

IMPACT/TECH TRANSFER A: Persistent organic pollutants such as polychlorinated dioxins, furans, and biphenyls enter the food chain as animals are exposed through their surroundings and feed. These toxic contaminants are concentrated in animal products containing fats (i.e. meat, dairy, and eggs) and are ultimately consumed by humans. In order to minimize our exposure from foods, the intake of these contaminants by animals must be reduced or their body burdens decreased before market. Knowledge of sources of contamination, such as PCP treated wood, are important to regulators and animal rearers so that measures may be taken to reduce the animal's intake. Minimizing the use of recycled PCP treated wood and posts in animal raising areas may be one method of reducing dioxin intake. Exploring mechanisms which can reduce existing body burdens is also of importance to animal scientists and regulators. Compounds which can increase metabolism and excretion of toxic contaminants may be used therapeutically in cases of extreme exposure. Information on basic metabolic processes may also be useful to scientists in defining the mechanisms of toxicity of these contaminants.

OBJECTIVE B: Determine whether nontoxic precursors or other environmental contaminants, such as pentachlorophenol, predioxins, or halogenated diphenyl ethers, are converted to more toxic compounds, including dioxins and furans, in mammalian systems.

PROGRESS B: The absorption, disposition, metabolism, and excretion of a nontoxic dioxin congener, 1,3,7,8-tetrachlorodibenzo-*p*-dioxin (1,3,7,8-TCDD), was studied in a ruminating calf. Metabolites isolated from the calf included two NIH-shifted hydroxytetrachlorodioxins which have also been found in rats dosed with 1,3,7,8-TCDD. One of these hydroxylated compounds resembles a major metabolite of 2,3,7,8-TCDD which is considered less toxic than the parent compound but has been shown to have a strong affinity for the thyroid transport protein, transthyretin. Structural similarities between these metabolites and the highly toxic 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD) and evidence for the possible disruption of thyroid hormone transport warrant further toxicity studies on these compounds.

Brominated flame retardants including polybrominated diphenyl ethers and bisphenol-A are produced in large quantities and have recently become a concern due to their highly lipophilic nature and structural similarities to dioxins and polychlorinated biphenyls (PCBs). These similarities may indicate a propensity to bioaccumulate and/or produce toxic responses in organisms. We have conducted absorption, disposition, metabolism, and excretion studies in rats using tetrabromobisphenol-A (BPA) and 2,2'4,4'-pentabromodiphenyl ether (BDE-99) in order to assess bioaccumulation and possible adverse interactions in mammalian systems. Conventional and bile-duct cannulated rats were orally dosed with ^{14}C-labeled BPA or BDE-99. Urine, bile, and feces were collected at 24 h intervals for three days. Both compounds were absorbed to a large extent based on biliary excretion and tissue retention. BPA was almost entirely (92%) excreted through the feces within 72 h after dosing. On the other hand only 44% of the BDE-99 dose was excreted in 72 h. Major sites of deposition for BDE-99 were the skin (38% of the dose) and adipose tissues (4%). These studies confirm the bioavailability of these flame retardants and also the high propensity for BDEs to accumulate and persist in animal tissues.

IMPACT/TECH TRANSFER B: These metabolism studies are important to scientists and regulators in that they help to identify the formation of potentially toxic metabolites and to predict which chemicals display persistent tendencies. One newly recognized persistent class of chemicals are the brominated flame retardants. Our studies suggest that brominated diphenyl ethers can readily accumulate in animal tissues and that levels in the environment and food supply should be investigated. Metabolites with structural similarities to known toxic compounds or endocrine disruptors may require further work be done on elucidating modes of actions of these chemicals.

OBJECTIVE C: Develop inexpensive, rapid methods for the detection of dioxins and related compounds in foods.

PROGRESS C: An immunoaffinity column (IAC) generated from a monoclonal antibody provided by Dr. Larry Stanker, ARS, Food Animal Protection Research Laboratory, College Station, TX, has shown promise as a convenient cleanup for polychlorinated dibenzo-*p*-dioxins (PCDDs) from serum samples. Previous studies showed that serum could be directly applied to the column, several of the most toxic PCDDs bound to the IAC, and the congeners could be eluted by 50% acetone before analysis by high resolution gas chromatography-high resolution mass spectrometry. Recently we have explored optimization of the IAC methodology. By adding a prewashing step before applying the sample, the recovery of 2,3,7,8-TCDD increased from 16% to 83%. Samples of up to 25 ml of serum showed acceptable recoveries (36-50%) of the four most toxic congeners on a 0.5 ml column. The smaller column size reduced elution volumes and any interferences which may have come from the column itself. When applied to human serum samples provided by Dr. Donald Patterson. Jr, Center for Disease Control and Prevention, Atlanta, GA, six dioxin and furan congeners were quantitated within the 95% confidence limits of the method at the part-per-quadrillion (ppq) level. These six congeners accounted for almost 80% of the toxic equivalency of the sample due to dioxins.

Animal fat samples have been purified by various methods in order to assess the amount of cleanup needed for screening of dioxins by an antibody-based assay supplied by Hybrizyme, Corp. Fat samples which had previously been analyzed by standard EPA methods were purified through a rigorous cleanup procedure (EPA Method 1613) and also by several abbreviated procedures. These samples will be used to validate the screening assay in the near future. The antibody-based assay has been shown to have sensitivity for 2,3,7,8-TCDD in the 10-20 picogram range. We have successfully used the assay in our laboratory with standards solutions.

IMPACT/TECH TRANSFER C: Presently available analyses of dioxins in food are too costly for regulatory monitoring ($1000/sample). A simpler sample cleanup could lead to faster analysis with a reduction in cost. Our studies indicate immunoaffinity columns can provide a rapid yet accurate analysis of the most toxic dioxins at the ppq level. This technique will be of importance to regulatory agencies and other scientists for reducing the time and costs associated with dioxin analysis.

Another way to reduce the cost of analysis is to develop inexpensive screening assays which can give preliminary concentration data on samples. A CRADA with Hybrizyme Corp., Raleigh, NC, has been established to assess an antibody-based screening assay. If successfully applied to animal samples this screening assay may make more widespread monitoring of dioxins economically feasible.

PUBLICATIONS:

Hakk, H., G.L. Larsen, and V.J. Feil. 1999. The binding of 1,2,3,7,8-pentachlorodibenzo-p-dioxin metabolites with rat urinary and biliary carrier proteins. Organohalogen Compounds. (in press)

Hakk, H., G.L. Larsen, E. Klasson-Wehler, and U. Orn. 1999. Tissue disposition, excretion, and metabolism of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) in male Sprague-Dawley rats. Organohalogen Compounds. (in press)

Huwe, J.K. and V.J. Feil, 1999. Metabolism of 1,3,7,8-tetrachlorodibenzo-p-dioxin in a ruminating calf. Organohalogen Compounds. (in press)

Huwe, J.K., V.J. Feil, G.L. Larsen, and R.G. Zaylskie. 1999. Pentachlorophenol levels in beef adipose tissue as an indicator of treated-wood exposure. Organohalogen Compounds. (in press)

Huwe, J.K., V.J. Feil, R.G. Zaylskie, and T.O. Tiernan. 1999. An investigation of the in vivo formation of octachlorodibenzo-p-dioxin. Chemosphere. (in press)

Huwe, J.K., W.L. Shelver, D.G. Patterson, and W.E. Turner. 1999. Application of an immunoaffinity column to the isolation of PCDD/Fs from serum samples. Organohalogen Compounds. (in press)

Larsen, G.L. and V.J. Feil. 1999. Levels of chlorinated dioxin and furan contamination at various depths in pentachlorophenol treated wood. Organohalogen Compounds. (in press)

Larsen, G.L., H. Hakk,, E. Klasson-Wehler, U. Orn, and A. Bergman. 1999. Binding of 2,2',4,4',5-pentabromodiphenyl ether ... metabolites to mammalian urinary and biliary carrier proteins. Organohalogen Compounds. (in press)

Shelver, W.L. and J.K. Huwe. 1999. The application of immunoaffinity chromatography for the analysis of TCDD in bovine milk. J. Liq. Chromatogr. & Rel. Technol. 22:813-821.

Shelver, W.L., J.K. Huwe, L.H. Stanker, and G.L. Larsen. 1999. Comparison of the binding efficiency of PCDD/Fs in biomatrices using immunoaffinity columns generated from mono- and polyclonal Ab. Organohalogen Compounds. (in press)

PROCEEDINGS/ABSTRACTS

Feil, V.J. and G.L. Larsen. 1999. Dioxins in food from animal sources. Am. Chem. Soc. Abstr. 39:103-104.

ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION OF VETERINARY DRUGS AND MYCOTOXINS IN FOOD ANIMALS

ARS Contact Persons: **D.J. Smith, N.W. Shappell, W. Shelver, G.L. Larsen**

CRIS NUMBER: 5442-32000-007
FSIS CODE: F1CA03
CRIS TERM. DATE February 2004

Biosciences Research Laboratory
Red River Valley Agricultural Research Center
Fargo, ND
Phone: 701-239-1238
Fax: 701-239-1430
E-mail: smith@fargo.ars.usda.gov

OBJECTIVE A: Determine the metabolism, distribution, excretion, and elimination properties of beta-adrenergic agonists in food producing animals.

PROGRESS A: Human food poisoning has been documented to have occurred after the consumption of clenbuterol-contaminated beef and pork. No residue depletion data exists for clenbuterol in swine, therefore a study was conducted to evaluate the depletion of total radioactive residues, parent clenbuterol, and clenbuterol stereoisomers in edible and non-edible tissues of swine. Eighteen cross-bred hogs (9 barrows and 9 gilts; 23.4 ±1.0 kg), housed in metabolism crates, were fed diets containing 1.0 ppm [^{14}C]clenbuterol for seven consecutive days. Six hogs (3 of each sex) were slaughtered after withdrawal periods of 0, 3, or 7 days. Excreta were collected during the course of the study in order to evaluate the disposition of radioactivity in the hogs. Tissues were collected at slaughter and analyzed for total radiocarbon content. Liver, kidney, and lung were evaluated for their content of parent clenbuterol and clenbuterol stereoisomers. Total radioactive residues (ppb) for edible tissues and lung are shown below:

Tissue	Sex	Withdrawal Period (Days)		
		0	3	7
Liver	♂	301 ± 6	31.4 ± 3.6	14.9 ± 2.3
	♀	270 ± 24	31.8 ± 2.5	14.6 ± 1.9
Kidney	♂	117 ± 26	9.9 ± 1.7	5.3 ± 0.5
	♀	107 ± 13	9.5 ± 2.6	4.6 ± 0.6
Muscle	♂	17.1 ± 2.5	1.8 ± 0.3	1.1 ± 1.2
	♀	17.5 ± 1.2	1.8 ± 0.3	0.9 ± 0.2
Adipose Tissue	♂	23.6 ± 12.9	10.5 ± 8.4	5.5 ± 1.2
	♀	20.7 ± 4.6	7.6 ± 4.0	5.0 ± 1.9
Lung	♂	314 ± 95	14.8 ± 7.0	4.8 ± 1.1
	♀	253 ± 58	13.6 ± 2.0	3.4 ± 0.2

Parent clenbuterol, as a percentage of total radioactive residues, decreased with increasing withdrawal period in liver, representing about 50, 40, and 15% of total radioactive residues at 0, 3, and 7 day withdrawal periods respectively. Parent clenbuterol residues in kidneys represented approximately 60% of the total residues at 0 withdrawal. Analyses of parent clenbuterol in other edible tissues and of the stereochemical composition of clenbuterol residues are in progress. In Europe, the tolerance for clenbuterol in livers of beef is 0.5 ppb which requires a withdrawal period of 28 days after treatment.

IMPACT/TECH TRANSFER A: This study provides basic data on the disposition of an illegally-used feed additive in a target species for illegal use. Elimination characteristics of the drug can be compared with target species such as cattle and horses, and generalizations regarding species differences may be inferred.

OBJECTIVE B: Evaluation of Mexican swine and cattle feed samples for the presence of clenbuterol HCl.

PROGRESS B: The analytical method developed by Blanchflower et al. (1993) for the determination of clenbuterol in tissues and urine was adapted and validated for the determination of clenbuterol in cattle and swine premix samples. Premix samples were obtained by collaborators in Nuevo León, Mexico, were extracted into ether and were derivatized with methyl boronic acid according to the procedure of Blanchflower et al. (1993; Biological Mass Spectrometry, 22:326-330). Electron impact mass spectra of a predominant peak present in total ion chromatograms of premix extracts were identical to those obtained from clenbuterol fortified water and feed samples. Mass spectra obtained from clenbuterol fortified and premix samples contained molecular and fragment ions consistent with those reported for the methyl boronic acid derivative of clenbuterol reported by Blanchflower et al. (1993). There was no evidence for the presence of clenbuterol in unfortified control samples. The assay was specific for clenbuterol and other b-adrenergic agents were not investigated. Cattle premix samples were positive for clenbuterol, but swine premix samples were negative. No claims are made by the analyst or by the USDA-Agricultural Research Service regarding the origin or source of the clenbuterol in the premix samples, nor does the analyst or the USDA-Agricultural Research Service have any knowledge of individual(s), company(ies), or organization(s) who fortified the analyzed feed substances with clenbuterol. The analyzed samples were not collected by the USDA for regulatory or legal purposes.

IMPACT/TECH TRANSFER B: The results document that clenbuterol is being used illegally in cattle feed in countries that border the United States. Copies of reports of the analyses have been shared with the US-FDA-CVM, the USDA-FSIS, and with the USDA-ARS-NPS.

OBJECTIVE C: To determine the cross reactivity of commercial clenbuterol immunoassays to purified clenbuterol metabolites and to clenbuterol stereoisomers.

PROGRESS C: Literature for commercially available immunoassay kits do not generally report cross-reactivities to metabolites or individual stereoisomers. However, some kits have been used for quantitative purposes. The presence of cross-reactive metabolites could compromise the accuracy of the kits if metabolites cross react to any appreciable degree or if stereospecificity existed for one stereoisomer over the other. Three clenbuterol-glucuronide conjugates, clenbuterol-sulphamate, 4-amino-3,5-dichloro-hippuric acid (clenbuterol hippurate), and purified clenbuterol stereoisomers were used to test their reactivities to 3 commercially available clenbuterol immunoassays. Clenbuterol sulphamate showed considerable cross reactivity among each of the three kits (42-77% of parent clenbuterol). The clenbuterol hippurate metabolite showed less than 0.2% cross reactivity to each kit and cross reactivities of clenbuterol glucuronide conjugates ranged from 0.1 to 1.6%. Two of the three kits showed no stereospecificity for clenbuterol, however one kit had an affinity for the (*S*+) isomer approximately 100 times greater than that for the biologically active (*R*-) isomer of clenbuterol.

IMPACT/TECH TRANSFER C: The use of immunoassays for quantitative purposes in matrices which may contain clenbuterol metabolites, or an non-racemic mixture of stereoisomers, may result in inaccurate results. If immunoassays are to be used for quantitative purposes, they must be validated against known metabolites and should be tested for stereoselectivity. For qualitative purposes, cross reactivity with clenbuterol metabolites may increase the assay sensitivity.

OBJECTIVE D: To develop an immunoassay for the β-agonist ractopamine.

PROGRESS D: Ractopamine HCl is a β-adrenergic agonist which is an active "leanness-enhancing" agent in several economically important livestock species. Unlike other phenethanolamine β-agonists targeted for agricultural use, ractopamine contains two para-substituted phenolic groups at opposite ends of its "ethanolamine" backbone and, as a result, it cross reacts poorly with antibodies against other β-agonists such as clenbuterol. Polyclonal antibodies against ractopamine-hemigluterate-KLH were generated using New Zealand white rabbits. Antibodies showed sensitivity to ractopamine at the ppb level. The degree of cross reactivity with ractopamine-glucuronides, the major metabolite of ractopamine, varied considerably. For example glucuronides conjugated to the *RS* and *SR* stereoisomers of the butylamine phenol of ractopamine showed about 4% cross reactivity with the antibodies, but similar glucuronides of the *RR* and *SS* stereoisomers did not cross react at all. Glucuronides conjugated to the ethanolamine phenol of ractopamine showed about 26% cross reactivity. Cross reactivities towards bis-phenylalkylamine β-agonists such as dobutamine (33%), fenoterol (1.3%), and ritodrine (0.8%) were greater than cross reactivities towards β-agonists with branched N-alkyl substituents such as isoxsuprine (0.1%), isoproterenol (<0.01%), metaproterenol (<0.01%), and clenbuterol (<0.01%).

IMPACT/TECH TRANSFER D: Antibodies generated against ractopamine could be used to monitor for illegal use of this β-agonist. Should this β-agonist be approved by the US-FDA the, antibody could be used to detect use of ractopamine in organizations which may ban β-agonist use; for example, governing organizations for livestock shows, county and state fairs, and breeding organizations.

PUBLICATIONS:

Ricke, E. A., D. J. Smith, V. J. Feil, G. L. Larsen, and J. S. Caton. 1999. Effects of Ractopamine HCl Stereoisomers on Growth, Nitrogen Retention, and Carcass Composition in Rats. J. Anim. Sci. 77:701-707.

Shappell, N. W., V. J. Feil, D. J. Smith, G. L. Larsen, and D. C. McFarland. 1999. Response of C_2C_{12} mouse and turkey cells to the b-adrenergic agonist ractopamine. J. Anim. Sci. (in press)

Smith D. J. 1998. Total Radioactive and Clenbuterol Residues in Edible Tissues, and the Stereochemical Composition of Clenbuterol Livers of Broilers after Exposure to Three Levels of Dietary [^{14}C]Clenbuterol HCl and Three Pre-Slaughter Withdrawal Periods. J. Anim. Sci. 76:3043-3053.

PROCEEDINGS/ABSTRACTS

Shelver, W. L. and D. J. Smith. 1999. Evaluation of commercial immunoassays for cross-reactivity to clenbuterol stereoisomers and bovine metabolites. Presented at the American Chemical Society Annual Spring Meetings, Anaheim, CA, Picogram No. 56:Abstract no. 63.

Smith, D. J. and M. Buchenau. 1999. Analysis of commercial premixes and feeds for the presence of b-adrenergic agonists. Presented at the American Chemical Society Annual Spring Meetings, Anaheim, CA, 1999. Picogram No. 56:Abstract no. 62.

PREVENT THE OCCURRENCE OR TOXINS IN WATER TO PROTECT FOOD AND THE ENVIRONMENT

ARS Contact Persons:
G.L. Larsen, E.A.E. Garber, H. Hakk, N.W. Shappell

CRIS NUMBER: 5442-42000-003
FSIS CODE: F1CC01,02
CRIS TERM. DATE: November 2003

Biosciences Research Laboratory
Red River Valley Agricultural Research Center
Fargo, ND
Phone: 701-239-1231
Fax: 701-239-1430
E-mail: larseng@fargo.ars.usda.gov

OBJECTIVE A: Identify and quantify biologically active compounds (BACs, e.g. toxins, mycotoxins, phytoestrogens, estrogens, or other endocrine-disrupting materials) in water, which may occur from a variety of known or unknown sources, 1) but associated with amphibian declines and deformities or 2) which may result from animal feeding operations, or 3) from municipal sewage treatment plants.

PROGRESS A: We have obtained water samples from lakes, wetlands and ponds in which deformed frogs have been found in Minnesota and Vermont. Collaborations have been established with researchers at the Minnesota Dept. Pollution Control/Vermont Dept. Natural Resources. Several personnel have obtained the training needed to conduct the bioassay (frog embryo teratogenesis assay - *Xenopus*, FETAX), which is to be used as a bioassay in this research and is now in the process of being set up. Preliminary work has begun on fractions from a C-18 solid phase extraction of water from affected and non-affected sites to isolate toxins present in the affected water responsible for the frog malformation. A study is being conducted in Vermont on mortality and deformities in larvae from eggs collected from both affected and non-affected sites and then reared in affected and non-affected sites.

Additional water samples will be obtained from manure composting and stockpiling sites and sites near municipal sewage treatment plants. These samples will be studied for estrogen levels to determine estrogen loading of the environment. Also, samples will be obtained from lakes, rivers, municipal water supplies, and sewage treatment facilities to determine levels of pharmaceuticals in the environment.

IMPACT/TECH TRANSFER A: These studies are important to the public, scientists and regulators because they identify potentially toxic chemical or chemicals present in water and which may ultimately contaminate food. Malformations in frogs are considered to be important because of their role as indicators, "sentinel species" of environmental health. This classification as an ecological indicator is partially based on the fact that they develop rapidly in water and live both in water and on land, which leave them particularly susceptible to environmental contaminants. Evidence also exists that estrogenic chemicals are present in the aquatic environment at concentrations high enough to adversely affect aquatic organisms. This research is significant because of a lack of knowledge regarding the health risks posed by these contaminants to people, wildlife, and aquatic ecosystems and their presence in the food or food products we consume.

ALTERNATIVE SOLVENT SYSTEMS AND TECHNIQUES FOR FOOD ANALYSIS (Residue Analysis)

ARS Contact Persons:
J.W. King, J.M. Snyder,
F.J. Eller, S.L. Taylor
J.A. Teel

CRIS NUMBER: 3620-45250-001
FSIS CODE: P1CC01
CRIS TERM. DATE: December 2003

Food Quality and Safety Research Unit
National Center for Agricultural Utilization Research
Peoria, IL
Phone: 309-681-6203
Fax: 309-681-6686
E-mail: kingjw@mail.ncaur.usda.gov

OBJECTIVE A: Utilization of binary fluid mixtures for trace residue analysis.

PROGRESS A: Continuation of previous studies using combinations of binary fluids for the selective extraction of pesticide residues from meats and other food products is being conducted in collaboration with the FDA Total Diet and Pesticide Research Center. To aid in this effort, three binary fluid generation systems were constructed at NCAUR, and one loaned to TDRC in Lenexa, Kansas. The range of candidate fluids has been expanded to include mixtures of carbon dioxide with R-134A. Both fortified butter and a variety of food matrices containing incurred residues were extracted and the analyte specificity of the extraction evaluated by measuring the recovery of pesticides as well as the coextracted lipid matter. Analyte recovery studies on the spiked butter samples were conducted down to 1 ppb levels and showed a decreasing (but not serious) reduction in recovery for both organochlorine- and organophosphorus-pesticides. Better results (>90% recovery) were achieved with the organophosphorus pesticides relative to the organochlorine pesticides. Using 70:30%/CO_2:N_2 and 75:25%/CO_2:R-134A mixtures, very successful recoveries of sub-ppb incurred residues from such matrices as tortilla chips, fish sticks, and crackers, were achieved.

IMPACT/TECH TRANSFER A: The binary fluid technology is being transferred to an FDA laboratory for further verification of its utility. Considerable information was also provided to Marvin Hopper of TDRC for use in FDA's International Total Diet Workshop, held on July 26-August 6, 1999 in FDA's Kansas City District Laboratory. NCAUR's critical fluid technology team's role in developing analytical methodology based on critical fluids was heavily cited.

OBJECTIVE B: Development of new alternative solvents and methodology for residue analysis.

PROGRESS B: A new CRIS proposal was written, successfully peer-reviewed and evaluated this past reporting period, based on input from FSIS. To further facilitate studies using compressed water as an extraction medium, the Lead Scientist undertook a sabbatical for six months at the University of Leeds's School of Chemistry, studying laboratory techniques relevant for conducting compressed water extractions. A postdoctoral research associate has been recently hired to conduct studies in this area,

and an accelerated solvent extraction module purchased for use in conducting extractions with heated compressed water.

IMPACT/TECH TRANSFER B: Two visits were made to the FSIS Midwestern Laboratory in St. Louis, Missouri to coordinate our research effort with their needs. Contact is being maintained with a potential CRADA partner, Applied Separations Inc. who continue to express interest in the NCAUR-constructed subcritical water extraction unit. A visit was made to the FDA's St. Louis Laboratory regarding their interest in application of these new methods for the characterization and analysis of herbal and natural products entering the commercial market.

PUBLICATIONS:

Hawthorne, S.B. and J.W. King. 1999. Principles and practice of analytical SFE. *In:* Practical Supercritical Fluid Chromatography and Extraction, M. Caude and D. Thiebaut (eds.), Harwood Academic Publishers, Chur, Switzerland, pp 219-282.

King, J.W. 1999. SFC in the food industry. In Encyclopedia of Separation Science, Academic Press Ltd., London. (in press)

King, J.W. 2000. The use of supercritical fluids in analytical chemistry. *In:* Chromatography - A Century of Discovery - 1900-2000. The Bridge to the Sciences and Technology, C.W. Gehrke, R.L. Wixom, and E. Bayer, Elsevier Scientific Publishing Company, Amsterdam, (in press)

Snyder, J.M., J. W. King, and Z. Zhang. 1998. Comparison of volatile analysis of lipid-containing and meat matrices by solid phase micro- and supercritical fluid extraction. *In:* Challanges in Isolation and Characterization of Flavor Compounds, C.J. Mussinan and M.J. Morello (eds), American Chemical Society, Washington, DC, pp. 107-115.

ABSTRACTS:

Eller, F.J. and J. W. King. 1999. SFE of fat: reconciliation of gravimetric and GC-FAME determinations. Abstracts of Pittcon 1999. Abstract #593.

Snyder, J.M., M. L. Hopper, and J. W. King. 1999. Extraction and enrichment of pesticides for analysis by using binary supercritical fluid mixtures. Abstracts - 217th National ACS Meeting, Division of Agrochemicals, Abstract #86

ALTERNATIVE SOLVENT SYSTEMS AND TECHNIQUES FOR FOOD ANALYSIS
(Nutrient Analysis)

ARS Contact Persons:
J.W. King, F.J. Eller,
J.M. Snyder, S.L. Taylor
J.A. Teel

CRIS NUMBER: 3620-45250-001
FSIS CODE: P1CC02
CRIS TERM. DATE: December, 2003

Food Quality and Safety Research Unit
National Center for Agricultural Utilization Research
Peoria, IL
Phone: 309-681-6203
Fax: 309-681-6686
E-mail: kingjw@mail.ncaur.usda.gov

OBJECTIVE A: Determination of fat soluble vitamins in foods using lipases and supercritical carbon dioxide (SC-CO_2) extraction/reaction.

PROGRESS A: With the assistance of a visiting Ph.D. candidate, Charlotta Turner, from the University of Lund in Sweden, a method is being developed which incorporates an enzymatic-catalyzed reaction in the presence of SC-CO_2 to facilitate the analysis of vitamin E and A in various food matrices. The enzyme, Novozyme 435, can hydrolyze and/or transesterify the vitamins, which are extracted and solubilized in SC-CO_2 from the food matrix. Initial studies have shown that by using the enzyme, recoveries greater than 80% can be achieved for both vitamins from a powdered milk matrix. Additional studies are being conducted to see under what conditions either or both hydrolysis or esterification can be optimized. The role of matrix water content is also being evaluated in terms of its effect on enzyme activity. Several types of model lipid compounds, such as pure triglycerides, are being used as diagnostic aids in optimizing the reaction/extraction step. In addition to milk, samples consisting of liver paste, minced meat, and baby food will also be extracted/reacted and assayed for their Vitamin E and A content.

IMPACT/TECH TRANSFER A: The study will aid in the development of reduced solvent methods for vitamin assay in food matrices, including a European Union-based collaborative method involving the application of supercritical fluid extraction (SFE) for vitamin analysis.

OBJECTIVE B: Analysis of sterols using analytical supercritical fluid techniques.

PROGRESS B: To supplement previously reported fractionation studies, enzyme initiated esterification reactions in supercritical carbon dioxide (SC-CO2) have been conducted on pure sterols (cholesterol and sitostanol) as an aid in analyzing similar moieties in foods. Four candidate enzymes were tested with respect to catalyzing the esterification, and Chirazyme L-1 was found most satisfactory for this purpose. Both static and continuous synthesis were utilized incorporating pressures from 3000-4500 psi, 40-60°C, a four- fold variation in flow rate, and various static hold times. Static hold time was

found to be crucial to achieving a high conversion (>90%), while both flow and static synthesis yield were sensitive to the chain length of the reacting alcohol. Recently, HPLC capabilities have been implemented for analyzing sterols, steryl esters, tocopherols, and similar nutritionally-active components in food matrices and agricultural extracts.

IMPACT/TECH TRANSFER B: The ability to separate and measure the amount of sterols in fat-containing foods is important to producers and consumers. concerned with the nutritional content of their products (e.g., Benecol). This study indicates that it is feasible to conduct a reaction in SC-CO_2 to form steryl esters which can aid in the analysis of sterol-enriched foods. Conversely, the results from these studies can also be used to synthesize steryl esters for subsequent incorporation into foodstuffs.

PUBLICATIONS:

Eller, F.J. and J.W. King. 1998. Supercritical CO_2 extraction of fat: Comparison of gravimetric and GC-FAME methods. J. Agric. Food Chem. 46:3657-3661.

Eller, F.J. 1999. Interference by methyl levulinate in determination of total fat by GC-FAME analysis of low-fat/high sugar products by gas chromatographic fatty acid methyl ester (GC-FAME) analysis. J. Assoc. Off. Anal. Chem. 82:766-769.

King, J.W. and F.J. Eller. 1999. Improved tools for the analytical chemist. Food Test Anal. 5(1):21-22,37.

Snyder, J.M, J.W. King, S.L. Taylor, and A.L. Neese. 1999. Concentration of phytosterols for analysis by supercritical fluid extraction. J. Am. Oil Chem. Soc. 76:717-721.

POISONOUS PLANT TOXINS AND THEIR EFFECTS ON LIVESTOCK

ARS Contact Person:
L.F. James
D.R. Gardner, S.T. Lee,
K.E. Panter, B.L. Stegelmeier

Poisonous Plant Research Laboratory
1150 East 1400 North
Logan, UT 84341
Phone: 435-752-2941
Fax: 435-753-5681
E-mail: lfjpprl@cc.usu.edu

CRIS NUMBERS: 5428-31320-002/32000-008/32630-008
FSIS CODE:
CRIS TERM. DATE: July 2002

OBJECTIVE: Develop detection techniques and identify plant toxins in feeds, foods, and animal products or tissues. Determine the toxicokinetics (absorption, distribution, excretion and clearance) of plant toxins from the tissues or products of animals that consume poisonous plants.

The mission of the Poisonous Plant Research Laboratory is to identify toxic plants, isolate and identify plant toxins, determine the mechanism of toxicity, document toxin metabolism and clearance from tissues, develop diagnostic and prognostic procedures, identify conditions of poisoning, and develop management strategies, antidotes, treatments and other recommendations to reduce losses, ensure product quality and promote animal and human health.

PROGRESS: Locoweeds (certain *Astragalus* and *Oxytropis* spp.) contain the toxin swainsonine. Swainsonine is rapidly absorbed and appears in all tissues and is excreted in milk and urine. Some tissues such as liver, pancreas and kidney accumulate high swainsonine concentrations (≥3000 ng/gm): this is nearly 10 times higher than serum concentration (generally 250 ng/gm). Swainsonine clearance (T½) from the serum, skeletal muscle, heart and spleen is rapid (T ½ ~ 20 hours) in sheep and cattle, while swainsonine clearance from the liver, kidney and pancreas is slower, T½ 60 hours. These results support recent findings suggesting that animals exposed to locoweed should be allowed a withdrawal time of about 28 days (10 half lives) to ensure swainsonine-free animal products. Because of the relatively short serum T½, use of swainsonine detection in blood as a diagnostic tool is limited. Even though poisoned animals may show severe clinical signs, if they have not eaten locoweed within a few days, no swainsonine may be detected.

More than 350 pyrrolizidine alkaloids from over 6,000 species of the Boraginaciea, Compositae and Leguminosae families are found throughout the world, and they often contaminate feeds and food. In a collaborative effort with the Natural Toxins Section of Australia's CSIRO, immunologic diagnostic techniques are being developed to quickly monitor feeds and food for possible contamination. Pyrrolizidine alkaloids form relatively stable pyrrole adducts with tissue proteins and nucleic acids. Chemical techniques have been developed to detect these pyrroles in animal tissues. Little is known about the clearance, ultimate fate, or toxicity of these adducts that could contaminate tissues. Current

research objectives are to determine pyrrole kinetics, pyrrole toxicity, and the effects of low pyrrolizidine exposures on fetuses and neonates.

Some lupine species contain quinolizidine and piperidine alkaloids that are toxic and teratogenic. Although these alkaloids have not been studied in animal tissues at this time, the blood absorption and elimination patterns of some of the key alkaloids have been determined in poisoned animals. Additional studies are needed to better define the toxicokinetics and risks of these alkaloids in animal products.

Larkspur (*Delphinium* spp.) poisons thousands of animals yearly; many more animals ingest less and do not appear to be clinically affected. These animals may have larkspur alkaloids present in many tissues although believed not to be a threat to humans because of feeding management practices and time before slaughter. The most toxic alkaloids have been identified and their structure activity characterized. Preliminary work determined that a key toxic alkaloid (methyllycaconitine, MLA) has a serum T½ of about 20 hours. Larkspur alkaloid conjugates have been produced and immune-based assays are being developed for rapid detection of toxic alkaloids in tissues and plants for diagnostic purposes. Additional work is underway to better define absorption, tissue distribution, excretion and clearance of larkspur alkaloids.

IMPACT/TECH TRANSFER: Research accomplished at the Poisonous Plant Research Laboratory has resulted in information, management strategies, diagnostic techniques and treatments for many poisonous plant problems. These results are important to the survival of rural ranching and small communities in many states. This work has increased producer and consumer knowledge of plant toxins, improved the technology to identify poisonous plant problems, improved animal productivity, and enhanced the utilization of pastures and rangelands where poisonous plants are found.

PUBLICATIONS:

James, L.F. 1999. Teratologic research at the USDA-ARS Poisonous Plant Research Laboratory. J. Nat. Toxins 8(1):63-80.

Panter, K.E., L.F. James and D.R. Gardner. 1999. Lupine, poison-hemlock and *Nicotiana* spp.: Toxicity and teratogenicity in livestock. J. Nat. Toxins 8(1):117-134.

Pfister, J.A., D.R. Gardner, K.E. Panter, G.D. Manners, M.H. Ralphs, B.L. Stegelmeier and T.K. Schoch. 1999. Larkspur (*Delphinium* spp.) poisoning in livestock. J. Nat. Toxins 8(1):81-94.

Stegelmeier, B.L., L.F. James, K.E. Panter, M.H. Ralphs, D.R. Gardner, R.J. Molyneux and J.A. Pfister. 1999. The pathogenesis and toxicokinetics of locoweed (*Astragalus* and *Oxytropis* spp.) J. Nat. Toxins 8(1):35-45.

Stegelmeier, B.L., J.A. Edgar, S.M. Colegate, D.R. Gardner, T.K. Schoch, R.A. Coulombe and R.J. Molyneux. 1999. Pyrrolizidine alkaloid plants, metabolism and toxicity. J. Nat. Toxins 8(1):95-116.

PART V: PATHOGEN CONTROL IN MANURE AND PRODUCE

MANAGEMENT OF HUMAN PATHOGENS IN MANURE, COMPOST AND IRRIGATION WATER

ARS Contact Persons:
R. E. Mandrell

CRIS NUMBER: 5325-41000-023
FSIS CODE: W1MC02
CRIS TERM. DATE: November 2002

Western Regional Research Center
Food Safety and Health
Albany, CA
Phone: 510-559-5829
Fax: 510-559-5948
E-mail: mandrell@pw.usda.gov

OBJECTIVE A: To develop methods for detecting, identifying and tracking human pathogens in manure, compost, water and bioaerosol environments related to fresh produce.

PROGRESS A: Strains of *E. coli* isolated from feed lot manure samples have been characterized extensively for virulence factors (shigaliketoxin activity, STX1, STX2, intimin, fliC and 60 Md plasmid), and O157 and H7 antigen expression. In a collaboration with a UC Berkeley scientist, a simple PCR assay has been developed for simultaneously identifying O157:H7 serotype strains, and fingerprint patterns for strain differentiation.

Plasmids optimized for expression of Green (GFP), Yellow (YFP), and Cyan Fluorescent Protein (CFP) in bacteria have been produced. Multiple *E. coli*, O157:H7, *Salmonella* sp., and *C. jejuni* fluorescent reporter strains have been produced and tested for stable expression of the plasmids and fluorescent proteins. Promoter libraries with fluorescent reporters are being constructed currently to determine by fluorescent activated cell sorting the genes highly regulated and important for survival in complex environments including manure and compost. The survival of human pathogens in manure, compost and water, and the resulting effect on attachment to, and survival in, plants will be determined.

OBJECTIVE B: To develop new methods for enriching, selecting and detecting bacterial pathogens from complex samples.

PROGRESS B: An immunofluorescence microscopy method for detection of *E. coli* O157:H7 in bovine manure has been developed in a preliminary study of pathogen antigen expression and biofilm formation in complex samples. Putative *E. coli* O157:H7 cells have been observed as aggregates or biofilms in culture positive samples of bovine feces.

IMPACT/TECH TRANSFER: The sensitivity of biosensors for detection of pathogens in complex samples will be directly dependent upon the methods for enriching the pathogen from the sample and presenting it in a form conducive for efficient detection in a biosensor or other method. Information on the physical state of pathogens in complex samples (e.g., suspended cells, aggregates/biofilms, intracellular) will be critical in the development of new sampling methods for sensitive detection of pathogens.

STABILIZATION AND SAFE USE OF MANURE AND MINERAL BY-PRODUCTS FROM RURAL/URBAN AREAS

ARS Contact Persons:
P.D. Millner, L.J. Sikora, J.S. Karns

CRIS NUMBER: 1270-120000-014
FSIS CODE:
CRIS TERM. DATE: June 2000

Soil Microbial Systems Laboratory
Room 140 Building 001 BARC-West
10300 Baltimore Ave.
Beltsville, MD
Phone: 301-504-8163
Fax: 301-504-8370
E-mail: pmillner@asrr.arsusda.gov

OBJECTIVE: To develop methods for the custom blending and treating of agricultural, industrial, and municipal by-products so that nutrients, pathogens, and odors are reduced and other undesirable factors are mitigated in the final product. Develop and evaluate appropriate usage practices for the products that are produced.

PROGRESS: Conducted training at the Scottish Agricultural College in Ayr. Training resulted in development of compost production process controlled by fuzzy logic to enhance the reliability of time-temperature exposures needed for pathogen destruction. Study was completed that recorded the change in P availability as poultry manure changed with time during composting. Silt loam or clay soils were amended with these composts to follow extractability with time. Final evaluation of data is underway. Tests for compost maturity and stability have shown that two tests, compost reheating test and the biological oxygen demand test are reliable tests for compost stability determinations relative to plant growth and soil conditioning. An important finding is that long term storage of samples at refrigerator temperatures does not affect the stability determination of the most immature or the most mature compost sample but that samples with maturities in between are affected by storage. Pathogen destruction by composting must be inferred from time-temperature criteria or by sampling and testing of treated products. Manures blended with basaltic rock dust lose less nitrogen to the atmosphere during composting than without rock dust, but rock dust does not stimulate the compost process as reported in the literature. Monthly field tests with N-Viro process equipment were conducted at the Beltsville Composting Research Facility. Cement kiln dust and coal combustion fly ash containing high amounts of calcium hydroxide and some calcium oxide were used to test the effectiveness of blend ratios with dairy, beef, and poultry manure. Initial results indicated that fecal coliforms and total bacteria were significantly reduced to very low levels within a few hours of treatment with all the blends that were stabilized at pH 11.0-11.5. In time course tests on all the treated manures, pH steadily dropped over 30 days to pH 10 in poultry and beef manure. The pH rapidly dropped over several days to pH 8.0-8.5 with dairy manure mixtures. Subsequent tests at higher total rates of alkaline byproduct additive (20%) were sufficient to reduce fecal

coliforms to undetectable levels and maintain pH above 10 for at least 30 days. No regrowth of fecal coliforms occurred in these specific trials. Baseline tests using GC/MS headspace analysis were conducted in collaboration with Laura McConnell (Environmental Chemistry Laboratory) to determine the type of odorous compounds present. Dimethyl disulfide, an intense and pervasive odorant, was present in treated and untreated dairy manure, but concentrations were greater in treated manure, although process operators and lab personnel could not detect the difference.

IMPACT/TECH TRANSFER: A patent application covering the use of a fuzzy logic control system for composting has been initiated. Compost maturity test results will be available to users as soon as the publications are completed and reviewed. The primary user will be the U. S. Composting Council that is currently compiling and evaluating compost test methods. The aggregates industry (stone, sand, and gravel) will obtain the results in the rock dust compost blend study and have an opportunity to comment on further studies. Fertilizer companies will be contacted for interest in the compost fertilizer blend results and possible CRADA development. Constraints are correct adoption of composting and blending techniques to obtain consistent products. Composts and composting can fail if guidelines for the process are ignored. During the next year, the process and product use test results, including pathogen destruction criteria, will be featured at two animal manure treatment technology transfer conferences open to public and private stakeholders. Presentations on the pathogen control portion of this work were made to the By-Products Uses Conference in Columbus, OH, the Composting Council Annual Conference in New Orleans, LA, and the American Society of Microbiology Annual Meeting in Chicago, IL.

PUBLICATIONS:

Sikora, L.J. and N. Enkiri. 1999. Fescue growth as affected by municipal compost fertilizers. Compost Sci. Util. 7:63-69.

Sikora, L.J.and N. Enkiri. 1999. Growth of tall fescue in compost/fertilizer blends. Soil Sci. 164:62-69.

FOOD ANIMALS, FOOD SAFETY, PUBIC HEALTH
PATHOGEN TRANSPORT AND DISSEMINATION FROM MANURE

ARS Contact Persons:
D. Shelton, R. Fayer, J. Higgins,
A. Isensee, G. McCarty
A. Sadeghi, J. Starr

CRIS NUMBER: 1265-32630-001
FSIS CODE:
CRIS TERM. DATE: October 2004

Immunology and Disease Resistance Laboratory (IDRL)
Livestock and Poultry Sciences Institute, ARS, USDA
Building 1040, Room 105
and Environmental Chemistry Laboratory (ECL)
Natural Resources Institute, ARS, USDA
Building 001, Room 210
Beltsville, Maryland 20705-2350
Phone: 301-504-6582 (ECL) -8201 (IDRL)
Fax: 301-504-5048 (ECL) -5306 (IDRL)
E-mail: dshelton@asrr.arsusda.gov

OBJECTIVE A: Determine rates and extent of pathogen transport/dispersal from bovine manures and survivability in pasture, crop and vegetable production systems.

PROGRESS A: This CRIS will be initiated October 1, 1999. Based on research from a previous related CRIS (1265-32000-001), it was determined that vertical transport of the protozoan parasite, *Cryptosporidium parvum* oocysts through soils is limited because oocysts adhere to or become entrapped by soil particles. After entrapment or adhesion oocysts rapidly decompose. These data indicate that once oocysts infiltrate into the soil profile the likelihood of leaching to groundwater, runoff to surface waters, or dissemination onto vegetation is remote. Similar studies are now in progress for bacteria.

IMPACT/TECH TRANSFER A:
This information is being disseminated to governmental action/regulatory agencies (e.g., EPA).

PUBLICATIONS:

Kuczynsk, E. and D.R. Shelton. 1999. Method for detection and enumeration of *Cryptosporidium parvum* oocysts in feces, manures, and soils. Appl. Environ. Microbiol. 65:2820-2826.

BIODEGRADATION AND THE BIOREMEDIATION OF AGROCHEMICALS IN SOIL

ARS Contact Persons:
J.S. Karns, W.W. Mulbry,
L.J. Sikora

CRIS NUMBER: 1270-120000-008
FSIS CODE:
CRIS TERM. DATE: September 1999

Soil Microbial Systems Laboratory
Room 140 Building 001 BARC-West
10300 Baltimore Ave.
Beltsville, MD
Phone: 301-504-6493
Fax: 301-504-8370
E-mail: jkarns@asrr.arsusda.gov

OBJECTIVE A: To control the distribution and movement of pathogens introduced into soil during agricultural operations through characterization of the biological, biochemical and molecular processes that affect them.

PROGRESS A: The survival of an *E. coli* O157:H7 strain in soils was examined. Manure addition was found to have no effect on the length of time the bacterium survived in any of the three soils tested. Growth of rye grass significantly lengthened the survival time of *E. coli* in all soils tested, particularly in the rhizosphere. In some soils the presence of alfalfa also lengthened the survival time. These experiments showed that agricultural practice can influence the survival of enteric pathogens associated with animal manure fertilizers when applied to soil. The downward movement of *E. coli* O157:H7 was characterized in three different soils. A large amount of the *E. coli* added were found to leach through soil columns, indicating that enteric pathogens added to soils with animal manures could contaminate shallow groundwater.

IMPACT/TECH TRANSFER A: Results of this work were presented at the BARC Poster Day and as a poster at the annual meeting of the American Society for Microbiology.

OBJECTIVE B: To develop practical method for the removal of nutrients and pathogens from the liquid fraction of animal manure treated by anaerobic digestion.

PROGRESS B: Preliminary experiments using an algal turf scrubber (ATS) system to treat digested dairy manure from Beltsville were completed. These experiments suggest that the ATS technology is capable of efficiently removing nitrogen and phosphorus from manure. The algal biomass produced contains about six percent nitrogen and one percent phosphorus after drying. Based on these results a pilot scale turf scrubber system was designed to generate enough algal biomass for animal feeding studies.

IMPACT/TECH TRANSFER B: Completion of laboratory-scale experiments has resulted in the planning of field-scale scrubbers for treatment of digested manure at Beltsville.

ABSTRACTS:

Gagliardi, J.G. and J.S. Karns. 1999. Leaching of O157:H7 through intact and repacked soil columns. 99th Annual Meeting of the American Society for Microbiology, Chicago, IL.

INTERVENTIONS TO IMPROVE THE MICROBIOLOGICAL SAFETY AND QUALITY OF FRUITS AND VEGETABLES

ARS Contact Persons:
K.B. Hicks, G.M. Sapers
C-H. Liao, W.F. Fett
B. Annous, D. Ukuku

CRIS NUMBER: 1935-41420-003
FSIS CODE:
CRIS TERM. DATE: September 2000

Eastern Regional Research Center
Plant Science & Technology Research Unit
Wyndmoor, PA. 19038
Phone: 215-233-6580
Fax: 215-233-6406
E-mail: khicks@arserrc.gov

OBJECTIVE A: Develop means of decontaminating fresh produce containing human pathogens.

PROGRESS A: The efficacy of commercial brush washing equipment, used in conjunction with conventional and experimental washing and sanitizing agents, in decontaminating apples was investigated in a commercial cidermill operated by the FDA in Placerville, California, with apples previously inoculated with a non-pathogenic *E. coli*. Brush washing did *not* significantly reduce the bacterial population on apples or in cider made therefrom with any washing agent tested, probably as a consequence of the inability of such washing systems to inactivate or remove microorganisms in inaccessible calyx and stem areas of apples. In other studies, a two-stage decontamination treatment for fresh apples entailing brief immersion in hot water followed by treatment with heated hydrogen peroxide solution showed promise. An agreement between ARS and Pennsylvania State University (PSU) was negotiated to facilitate the design, fabrication and evaluation of a BL-2 fruit and vegetable processing research facility. Plans for short-term research to develop new decontamination technology and solve design problems limiting further development of the system were initiated. Efforts are underway to sponsor a PSU faculty sabbatical at ERRC and to recruit a graduate cooperative education student to participate in the project. The frequency with which bacteria are found within internal tissues of fresh, unblemished apples is under investigation. Mature and immature apples from various locations are being examined. Progress is being made in development of treatments to reduce the population of human pathogens on external surfaces of cantaloupe.

IMPACT/TECH TRANSFER A: The inability of the commercial brush washer to reduce bacterial loads on apples demonstrates the need for new fruit washing technology that can overcome this limitation. Collaborative research in this area with Penn State and acquisition of a BSL-2 facility will give ARS a unique capability for improving the microbiological safety of fresh produce. Information on the efficacy of conventional and experimental washing formulations in decontaminating cider apples and on potential hazards in cider production was conveyed to growers.

packers, processors, the FDA and the scientific community through communications with the U.S. Apple Association and participation in meetings sponsored by the FDA (NCFST, CFSAN). The presence of human pathogens within the flesh of apples or other commodities, if demonstrated, would greatly limit the value of washing or other surface treatments as a means of decontaminating produce and also would be likely to affect future regulatory action aimed at improving the microbiological safety of produce. Studies of bacterial attachment to cantaloupe, survival and response to washing are providing a foundation for the development of treatments to assure the microbiological safety of this commodity.

OBJECTIVE B: Examine dynamics of interactions between spoilage microorganisms, foodborne human pathogens, and saprophytic epiphytes on fresh and minimally processed fruits and vegetables.

PROGRESS B: To determine whether areas of decay in grounder apples ("drops") may be a potential source of *E. coli* O157:H7 in apple cider made from such fruit, wounds in fresh apples were inoculated with this human pathogen and one of two decay fungi, *Penicillium expansum* or *Glomerella cingulata.* The former fungus was antagonistic to the bacteria and reduced their population. However, when apple wounds were inoculated with *Glomerella* and *E. coli* O157:H7 at the same time, extensive bacterial growth occurred, probably as a consequence of the decrease in acidity that results from fungal growth. Because the efficacy of washing as a means of decontaminating produce may be limited by the nature of bacterial attachment to produce surfaces, attachment of *Salmonella* to green pepper disks and apple fruits was investigated. Following inoculation, the distribution of these bacteria on various surfaces and ease of detachment was determined. *Salmonella* attached in greater numbers and survived washing to a greater extent on cut surfaces of pepper disks, compared to natural external or internal surfaces, and on the calyx and stem areas of apples, compared to the remaining skin surface. In other studies, firmness of attachment of non-pathogenic *E. coli* to external surface of cantaloupe increased as the interval between contamination and washing increased.

IMPACT/TECH TRANSFER B: Since the extent of growth of *E. coli* O157:H7 in the presence of *Glomerella* would be sufficient to contaminate a large quantity of cider, these results clearly demonstrate the potential risk of using decayed apples for production of unpasteurized cider. The results of bacterial attachment studies confirm the importance of bacterial attachment to inaccessible sites or cut surfaces (including punctures or other wounds) on produce and the time interval between contamination and packing/processing as factors limiting the efficacy of washing as a means of decontamination.

OBJECTIVE C: Develop means to improve the microbiological safety of sprouts.

PROGRESS C: Because of numerous outbreaks of food-borne illness associated with alfalfa sprouts and the inadequacy of current control methods, research on novel combination treatments for alfalfa seeds was carried out in collaboration with the Food Safety Research Unit at ERRC. A combination treatment entailing gamma irradiation followed by chemical sanitizing with 2% calcium

hypochlorite yielded a 5 log reduction in the population of *Salmonella* on inoculated seeds, a reduction level that could not be achieved with the individual treatments alone. The efficacy of 20,000 ppm chlorine in sanitizing seed contaminated with *E. coli* O157:H7 was confirmed. Studies on use of natural plant-derived extracts with anti-microbial activity have identified one extract that was as active as 2% calcium hypochlorite for sanitizing alfalfa seed. A research associate has been hired to continue research on the use of competitive exclusion to control growth of human pathogens in sprouts.

IMPACT/TECH TRANSFER C: The combination treatment strategy should enable sprout growers to achieve FDA goals with current technology if there are no unforeseen regulatory restrictions or economic constraints. Information on the microbiological safety of sprouted seeds and on the efficacy of intervention strategies was communicated to the FDA (CFSAN) and the International Sprout Growers Association. Data generated on use of 2% calcium hypochlorite was used in support of a successful joint USDA/industry petition to the EPA to allow use of this agent for sanitizing seed destined for sprouting. This technology is now widely used in the U.S. sprouting industry.

PUBLICATIONS:

Buchanan, R.L., S.G. Edelson, R.L. Miller, and G.M. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. Food Microbiol. 62: 444-450.

Fett, W.F., C. Wijey, R.A. Moreau, and S.F. Osman. 1999. Production of cutinase by *Thermomonospora fusca* ATCC 27730. J. Appl. Microbiol. 86: 561-568.

Janisiewicz, W.J., W.S. Conway, M. Brown, G.M. Sapers, P.Fratamico, and R.L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on fresh cut apple tissue and its transmission by fruit flies. Appl. Environ. Microbiol. 65: 1-5.

Liao, C.-H. and G.M. Sapers. 1999. Influence of soft rot bacteria on growth of *Listeria monocytogenes* on potato tuber slices. J. Food Prot. 62: 343-348.

Liao, C.-H., L. Revear, A. Hotchkiss, and B. Savary. 1999. Genetic and biochemical characterization of an exopolygalacturonase and a pectate lyase from *Yersinia enterocolitica*. Can. J. Microbiol. 45: 396-403.

Sapers, G.M., R.L. Miller, and A.M. Mattrazzo. 1999. Effectiveness of sanitizing agents in inactivating *Escherichia coli* in Golden Delicious apples. J. Food Sci. (in press)

Sapers, G.M., R.L. Miller, S-W. Choi, and P.H. Cooke. 1999. Structure andComposition of mushrooms as affected by hydrogen peroxide wash. J. Food Sci. (in press)

PROCEEDINGS/ABSTRACTS:

Liao, C.-H. 1999. Attachment and growth of *Salmonella chester* on apple fruits and *invivo* response of attached bacteria to sanitizer treatments. Proceedings of the 17th Int'l Conf. on Food Microbiol. And Hygiene. Veldhoven, The Netherlands.

Sapers, G. M., R. L. Miller, and A. M. Mattrazzo. 1999. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of cider apples. Annual Meeting of Institute of Food Technologists, Chicago, IL.

Rajkowski, K. T., Fett, W. F., and Thayer, D. 1999. Control of human pathogens on sprouts. 10th Annual Meeting of the International Sprout Growers Association, New Orleans.

QUALITY MAINTENANCE AND FOOD SAFETY OF FRESH AND FRESH-CUT FRUITS AND VEGETABLES

ARS Contact Persons:
K. Gross
B. Whitaker, J. McEvoy

Horticultural Crops Quality Laboratory
Plant Sciences Institute
Beltsville, MD
Phone: 301-504-6128
Fax: 301-504-5107
E-mail: kgross@asrr.arsusda.gov

CRIS NUMBER: 1275-42430-001
FSIS CODE:
CRIS TERM. DATE: July 2002

OBJECTIVE: To genetically engineer new lines of fruits and vegetables that are of superior quality and shelf-life thereby diminishing the threat of microbial safety problems and to identify postharvest storage conditions at which growth of human pathogens is restricted..

PROGRESS: Using gene knock-out techniques to reduce activity of the cell wall degrading enzyme β-galactosidase *in situ*, we have genetically-engineered superior tomato lines that produce fruit which soften more slowly, possibly reducing susceptibility to microorganism growth after harvest and during fresh-cut processing and marketing. Subsequent plant generations, as well as multiple gene knock-outs, are currently being evaluated. In addition, an optimum postharvest system was developed for storage of fresh-cut tomato slices using modified atmosphere packaging. Growth of *Salmonella* on fresh-cut slices under various storage conditions is currently being evaluated.

IMPACT/TECH TRANSFER: We are currently in CRADA negotiations with ZENECA Plant Sciences, Ltd. to cooperate on evaluation of our genetically-engineered tomato lines and for potential licensing of our patent for use of the genes for tomato quality improvement. Because shelf-life and quality are integral to microbial safety, such superior tomato lines should be excellent for use in producing fresh-cut tomato slices with a longer shelf-life and higher degree of microbial safety. Our results on an optimal packaging system for fresh-cut slices interfaces well with our genetic approach.

PUBLICATIONS:

Hong, J.H. and K.C. Gross. 1998. Surface sterilization of whole tomato fruit with sodium hypochlorite influences subsequent postharvest behavior of fresh-cut slices. Postharvest Biol Technol. 13:51-58.

Smith, D.L., D.A. Starrett and K.C. Gross. 1998. A gene coding for tomato fruit β-galactosidase II is expressed during tomato fruit ripening: Cloning, characterization and expression pattern. Plant Physiol. 117:417-423.

ABSTRACTS:

Hong, J.H. and K.C. Gross. 1999. Prolonging the shelf-life of fresh-cut tomato slices through modified atmosphere and low temperature. *HortScience*, Annual Meeting, Minneapolis, MN, p. 506.

Hong, J.H. and K.C. Gross. 1999. Ethylene inhibits the development of chilling injury in fresh-cut tomato slices during storage at low temperature. *HortScience*, Annual Meeting, Minneapolis, MN, p. 525.

Smith, D.L. and K.C. Gross. 1999. A family of seven β-galactosidase genes is expressed during tomato fruit development. *Plant Physiol.* p. 69.

PATENTS:

Gross, K.C. and D.L. Smith (1999) Genes coding for tomato β-galactosidase polypeptides. International Patent (Non-provisional) Application Number 60/088,805.

MICROBIOLOGICAL SAFETY OF FRESH FRUITS AND VEGETABLES

ARS Contact Persons:
K. Gross,
C. Bloch, A. Bhagwat

CRIS NUMBER: 1275-42000-002-00D
FSIS CODE:
CRIS TERM. DATE: December 2003

Horticultural Crops Quality Laboratory
Plant Sciences Institute
Beltsville, MD
Phone: 301-504-6128
Fax: 301-504-5107
E-mail: kgross@asrr.arsusda.gov

OBJECTIVE: To understand how human pathogens contaminate, survive and grow on fresh fruits and vegetables by understanding the host-pathogen interactions involved and then to use this information to devise new postharvest strategies to control or eliminate human pathogenic bacteria, particularly *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*, on fresh and fresh-cut fruits and vegetables.

PROGRESS: This is a new CRIS Project currently under development. Two Microbiologists are being hired. Their associated technical support positions should be filled by the end of 1999. Research should begin in the Fall of 1999.

REDUCTION IN FUNGICIDE USE BY DETERMINING ALTERNATIVE STRATEGIES TO CONTROL POSTHARVEST DECAY

ARS Contact Persons: **W.S. Conway, R.A. Saftner, J. McEvoy**

CRIS NUMBER: 1275-43000-004
FSIS CODE:
CRIS TERM. DATE: September 2000

Horticultural Crops Quality Laboratory
Plant Sciences Institute
Beltsville, MD
Phone: 301-504-6980
Fax: 301-504-5107
E-mail: wconway@asrr.arsusda.gov

OBJECTIVE: To determine the potential for foodborne human pathogens to survive and multiply on fresh-cut apple fruit.

PROGRESS: Populations of *Listeria monocytogenes* inoculated into decayed apple tissue continually increased on fruit decayed by *Glomerella cingulata* but did not survive after 5 days on fruit decayed by *Penicillium expansum.* The pH of the decayed area declined from pH 4.7 to 3.7 in the case of *P. expansum*, but in the case of *G. cingulata* the pH increased from ph 4.7 to 7.0. This pH modification may be responsible for affecting the growth of the foodborne pathogen.

IMPACT/TECH TRANSFER: This work contributes to the basic understanding of the potential for apples to become contaminated with *L. monocytogenes* and this problem may be especially acute in fruit stored for longer periods of time. This research provides information to the fresh produce industry by showing that proper storage temperature, as well as the absence of postharvest apple pathogens such as *G. cingulata,* is important for maintaining the safety of fresh-cut apples

PUBLICATIONS:

Janisiewicz, W.J., W.S. Conway, M.W. Brown, G.M. Sapers, P. Fratamico and R.L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. Appl. Environ. Microbiol. 65:1-5.

ABSTRACTS:

Conway, W.S., W.J. Janisiewicz, A.E. Watada and C.E. Sams. 1998. Survival and growth of *Listeria monocytogenes* on fresh-cut apples. Phytopathology 88 (Supplement):S18.

ADHESION OF HUMAN PATHOGENS TO SURFACES OF FRUITS AND VEGETABLES

ARS Contact Persons:
R. E. Mandrell, M Friedman, M. Wachtel, A. Charkowski

Western Regional Research Center
Food Safety and Health
Albany, CA
Phone: 510-559-5829
Fax: 510-559-5948
E-mail: mandrell@pw.usda.gov

CRIS NUMBER: 5325-41000-022
FSIS CODE: W1MC02
CRIS TERM. DATE: November 2000

OBJECTIVE A: To identify and describe molecular mechanisms of attachment of pathogens to surfaces of fruits and vegetables.

PROGRESS A: More than 50 strains of *E. coli* isolated from feed lot manure samples (~26,000) have been obtained and characterized for virulence factors (shigaliketoxin activity, STX1, STX2, intimin, fliC and 60 Md plasmid), and O157 and H7 antigen expression. In a collaboration with a UC Berkeley scientist, a simple PCR assay has been developed for simultaneously identifying O157:H7 serotype strains, and fingerprint patterns for strain differentiation.

Multiple environmental and clinical *E. coli* O157:H7, *Salmonella* and *C. jejuni* fluorescent reporter strains have been produced and used in studies of bacteria attachment to, and internalization in, lettuce, sprouts, cilantro and parsley. Time course studies of strains of *E. coli* O157:H7 and *Salmonella* in lettuce, sprouts, and cilantro have confirmed that pathogens survive well at 4C and increase in number up to 3 to 4 logs at 25C. Fluorescent microscopy studies indicate that pathogens localize to specific sites on surfaces, but also can migrate into veins of cut plants.The location of pathogens on plants have been identified by confocal microscopy. Promoter probe libraries are being produced for studies of environmental up-regulation of bacterial genes.

IMPACT/TECH TRANSFER A:
An understanding of the biology of human pathogens in pre-harvest and post-harvest plants is crucial for developing new control strategies. Simple methods for identifying pathogenic *E. coli* and genotypically related strains will be important in epidemiological analysis of outbreaks.

OBJECTIVE B: To identify new technology, including new compounds, that can minimize pathogens in foods.

PROGRESS B: *E. coli* O157:H7 has been shown to attach to lettuce surfaces, survive for up to 14 days on lettuce and internalize in the xylem. *Salmonella* has been shown to bind to specific locations

of alfalfa and other sprouts. Reporter strains have been produced for assays of attachment and potential attachment inhibitors. Plant extracts, essential oils and purified compounds have been screened for antimicrobial activity for pathogens. At least 10 compounds with potent activity have been identified. Biofilms on a variety of plant surfaces have been identified. Fitness studies of plant and human pathogens and plant epiphytes are currently in progress.

IMPACT/TECH TRANSFER B: Information on the attachment of pathogens in foods (e.g., single organism, biofilms, exterior/interior surface, etc.), and mechanism of pathogen binding to food surfaces will help producers develop and test strategies to control the amount of viable pathogen contamination and inhibitors of attachment, and/or compounds for controlling the amount of viable pathogen contamination.

ABSTRACTS:

Charkowski, A.O., S. Lindgren, and R.E. Mandrell. 1999. Investigation of *Salmonella enterica*-alfalfa sprout interactions. Annual Meeting American Phytopathol. Soc. Montreal, Canada.

Friedman, M., Improvement in the Quality and Safety of Food. Kansas City Section of the American Chemical Society, Kansas City, KS.

Friedman, M., F.F. Bautista, L.H. Stanker, and K. Larkin. 1999. Analysis of potato glycoalkaloids by a new ELISA kit. 82d Annual Meeting Potato Association of America, Fargo, ND.

Friedman, M., T.E. Fitch, C.E. Levin, and W.H. Yokoyama. 1999. Reduction of LDL cholesterol in hamsters fed green and red tomatoes. Division of Agricultural and Food Chemistry, Annual Meeting American Chemical Society, Boston, MA.

Wachtel, M.R., S.M. Leffel, and R.E. Mandrell. 1999. The use of the Green Fluorescent Protein in the study of bacterial pathogens: adherence of enterohemorrhagic *Escherichia coli* to lettuce leaf surfaces. Second International Symposium on the Green Fluorescent Protein, San Diego, CA.

Wachtel, M., S. Leffel, and R. Mandrell. 1999. Attachment of enterohemorrhagic *E. coli* serotype O157:H7 to lettuce leaf surfaces. 99th General Meeting American Society of Microbiology, Chicago, IL. Poster P-77.

FOOD SAFETY, WASTE MINIMIZATION, AND VALUE ENHANCEMENT OF FERMENTED AND LIGHTLY PROCESSED VEGETABLES

ARS Contact Persons:
H.P. Fleming, W.M. Walter, Jr.
R.F. McFeeters, F. Breidt

CRIS NUMBER: 6645-41420-002
FSIS CODE:
CRIS TERM. DATE: July 2003

Food Science Research Unit
Raleigh, NC
Phone: 919-515-2979
Fax: 919-856-4361
E-mail: hfleming@unity.ncsu.edu

OBJECTIVE A: To develop methods to prevent growth of pathogenic and spoilage microorganisms in minimally preserved, brined, and fresh-cut refrigerated vegetables.

PROGRESS A: A mathematical model was developed which consists of a system of non-linear differential equations describing the growth of competing bacterial cell cultures. In this model, bacterial cell growth is limited by the accumulation of protonated lactic acid and decreasing pH. In the experimental system, growth of pure and mixed cultures of *Lactococcus lactis* and *Listeria monocytogenes* was carried out in a vegetable broth medium. Predictions of the model indicate that pH is the primary factor limiting the growth of *L. monocytogenes* in competition with a strain of *L. lactis*, which does not produce the bacteriocin nisin. The model also predicts the values of parameters affecting the growth and death of the competing populations. Further development of this model will incorporate the effects of additional inhibitors such as bacteriocins, and may aid in the selection of lactic acid bacteria cultures for use in the competitive inhibition of pathogens in minimally processed foods.

Blanching of whole cucumbers for 15 seconds at 80 degrees C was found to reduce microbial cell counts by three log cycles from an initial population of typically 10^6 CFU/g. Vegetative microorganisms survived this blanching process presumably because they were located beneath the surface of the cucumber. The sensitivity to heat for selected populations was measured by determining D values for pooled microorganisms (termed D_p values) isolated from fresh cucumbers. From these populations, we found that the total *Enterobacteriaceae* and the total aerobic bacteria had similar D_p values to each other and to a selected lactic acid bacterium. The addition of phytochemicals to minimally processed vegetable products is also being investigated. Allyl isothiocyanate (AITC) was found to have potential application in products where the flavor perception is compatible.

Methods to inactivate and prevent buildup of microorganisms in wash and hydrocool water are being investigated. Chlorine dioxide at 1.3 ppm was found to prevent buildup of microorganisms in recycled water used for hydrocooling whole, pickling cucumbers. Currently, we are investigating

the use of pulsed UV light to inactivate bacteria in water used for washing and hydrocooling of fresh vegetables.

IMPACT/TECH TRANSFER A: Recent results on our research have been published in scientific journals. The mathematical modeling of biocontrol and pathogenic bacteria for use in food systems is very timely and of considerable interest to food microbiologists worldwide. We anticipate that this research will lead to novel application for prevention of food poisoning in fresh and minimally processed vegetables. The proper use of compatible phytochemicals to improve the safety and extend the storage life of minimally processed vegetable products is a reasonable expectation of this research. We have had numerous inquiries on the possible use of AITC in various food products. Some commercial farmers and processors have begun to use chlorine dioxide in their cucumber hydrocooling operations. Since the need for chlorine dioxide (1.3 ppm) for effectiveness is much less than that for chlorine (100-200 ppm), there is less concern for chlorine byproduct formation of health consequence. If our research on pulsed UV light proves economially feasible, even less concern for chemical byproducts will result.

PUBLICATIONS:

Breidt, F. and H.P. Fleming. 1998. Modeling of the Competitive Growth of *Listeria monocytogenes* and *Lactococcus lactis* in Vegetable Broth. Appl. Environ. Microbiol. 64:3159-3165.

Shofran, B.G., S.T. Purrington, F. Breidt, and H.P. Fleming. 1998. Antimicrobial Properties of Sinigrin and its Hydrolysis Products. J. Food Sci. 63:621-624.

ABSTRACTS:

Breidt, F., J.S. Hayes, and H.P. Fleming. 1999. Reduction of microflora on fresh vegetables by blanching. 99th American Society for Microbiology Meeting, Chicago, IL.

Plengvidhya, V., H.P. Fleming, and F. Breidt. 1999. A PCR method for identifying unmarked lactic acid bacteria starter cultures in vegetable fermentations. 99th American Society for Microbiology Meeting, Chicago, IL.

Yoon, S.S., F. Breidt, and H.P. Fleming. 1999. Occurrence and characterization of lactic acid bacteria bacteriophages from fermenting sauerkraut. 99th American Society for Microbiology Meeting, Chicago, IL.

PART VI: NEW AREAS OF ARS RESEARCH

MOLECULAR SYSTEMATICS AND DIAGNOSTICS FOR PARASITES OF FOOD AND FOOD ANIMALS

ARS Contact Persons:
E.P. Hoberg
B.M. Rosenthal

CRIS NUMBER: 1265-42000-002
FSIS CODE:
CRIS TERM. DATE: November 2003

Beltsville Area
Biosystematics and National Parasite Collection Unit
Beltsville, MD
Phone: 301 504-8530
Fax: 301 504-8979
E-mail: ehoberg@lpsi.barc.usda.gov

Objectives: (1) Determine molecular characters for strains/ genotype identification and diagnosis of *Toxoplasma, Cryptosporidium, Cyclospora* and other parasites of importance as food or waterborne pathogens, or which otherwise threaten food products or reduce production efficiency.

Progress: This is a new project. A new Scientist, Benjamin M. Rosenthal, has been recruited; EOD August 30, 1999. A new laboratory is currently being furnished and equipped.

DEVELOP IMAGING TECHNOLOGY FOR THE INSPECTION OF POULTRY PRODUCTS

ARS Contact Persons:	CRIS NUMBER:	6612-42000-030
C. E. Lyon,	FSIS CODE:	
W. R. Windham,	CRIS TERM. DATE:	2004
K. C. Lawrence		

Russell Research Center
Poultry Processing & Meat Quality Research Unit
Athens, GA
Phone: 706-546-3513
Fax: 706-546-3633
E-mail: rwindham@ars.usda.gov

OBJECTIVE A: To evaluate the feasibility of hyperspectral imaging and other technologies for identification of fecal and crop ingesta surface contamination on poultry carcasses and to develop a real time on-line imaging system for contaminate identification.

PROGRESS A: This research was iniated as a new 2 SY CRIS in April 1999. A hyperspectral imaging system, consisting of a visible / near infrared spectrograph and a charged coupled device camera has been built for use in the laboratory to image poultry carcasses contaminated with fecal material. Calibration protocols have been developed and preliminary studies including lighting and camera control have been designed.

IMPACT/TECH TRANSFER A: A Specific Cooperative Agreement (SCA) has been signed with the Institute for Technology Development, John C. Stennis Space Center, MS. on hyperspectral imaging techniques for inspection of poultry carcasses. An additional SCA has been signed with the Biological and Agricultural Engineering Dept., University of Georgia, to characterize and determine optimum operating conditions of the hyperspectral imaging system. Under this Agreement, Dr. B. Park has been hired as a Research Associate.

NEW TECHNOLOGY AND SYSTEMS TO DETECT AND PREVENT CHEMICAL AND MICROBIAL FOOD CONTAMINANTS

ARS Contact Person:
Shu-I Tu

CRIS NUMBER: 1935-42000-035
FSIS CODE:
CRIS TERM. DATE: 02/01/04

Eastern Regional Research Center
Microbial Biophysics Biochemistry Research Unit
Wyndmoor, PA 19038
Phone: 215-233-6611
Fax: 215-233-6581
E-mail: stu@arserrc.gov

Purdue University Contact Person:
Richard Linton, Food Safety Engineering Project Director
Randy Woodson, Director of Agriculture Research Programs
1140 Agricultural Administration Bld, Agricultural Research Programs
Purdue University
West Lafayette, IN 47907-1140
E-mail: linton@purdue.edu

OBJECTIVES: To develop a food safety engineering research project focusing on four objectives which are to:

1. develop diagnostic tools for rapid identification of biological and chemical foodborne contaminants
2. develop models to predict and track foodborne contaminants
3. identify, design, and evaluate alternative processing, handling, packaging, transport, and storage systems to minimize and/or reduce food contaminants
4. develop technology transfer of information and knowledge related to food safety for the food industry, government agencies, academia, and the public

PROGRESS: A request for proposals (RFP) process was developed based on four different funding categories. A description of the four different funding categories is listed on the following page and project awards are listed following the RFP category descriptions.

Category 1: Development of diagnostic tools for rapid identification of biological and chemical foodborne contaminants. This category supports the development of biochemical, chemical, and physical diagnostic tools or "markers" that can be used to identify biological and chemical foodborne contaminants. Projects will be developed that utilize current or new technologies that can be used to identify low levels of foodborne contaminants within food systems accurately and rapidly.

Priority will be given to projects that support the development of diagnostic tools to identify emerging foodborne contaminants that are difficult to identify, and for those contaminants that pose a severe threat to human health.

Category 2: Development of models to predict and track foodborne contaminants. This category supports the development of sensors that can be used to detect "markers" of biological and chemical contaminants in foods. Development of this technology should provide information on the growth, death, survivability, and/or presence of food contaminants. This category will also support the development of models to track foodborne contaminants in different areas of food production including the farm, transportation, processing, retail markets, and consumers. Priority will be given to those projects that model contaminants that pose a severe threat to human health, and for those projects that tract foodborne contaminants for multiple areas of food production, processing, and preparation.

Category 3: Identification, design and evaluation of alternative processing, handling, packaging, transport, and storage systems to minimize and/or reduce food contaminants. This category supports the development of new methods and improvement of existing methods to reduce biological and chemical contaminants in food systems. Priority will be given to those projects that use methods to reduce contaminants that pose a severe threat to human health and for procedures that can be directly applied to food systems.

Category 4: Development of technology transfer of information and knowledge related to food safety for the food industry, government agencies, academia, and the public. This category supports the development of technology transfer programs for emerging and important food safety issues. Technology transfer programs may include development of workshops and conferences or the development of computer accessible information. Priority will be given to those projects that develop technology transfer programs that support the research-focused categories.

After a peer review process, five projects were awarded. Four projects were researched focused and one project was technology transfer focused. Project titles and investigators are:

Biosensor-based approaches for rapid and sensitive detection of *Listeria monocytogenes* from food:A. Bhunia and M. Morgan

Detection of *Fusarium* species in grains and foods by ELISA & PCR: M. Cousin and C. Woloshuk

Alternative technologies for elimination of foodborne pathogens in fruits, vegetables and sprouts: R. Singh Bhunia, R. Stroshine and J. Simon

Food Safety Engineering information dissemination system: J. Marks

Tracking and predicting cases of human salmonellosis from *S. enteritidis* food poisoning: M. Saeed

AGRICULTURAL RESEARCH SERVICE RESEARCH LABORATORIES AND SCIENTISTS WORKING ON FOOD SAFETY INCLUDING THOSE RESPONDING TO FSIS RESEARCH NEEDS

ARS Liaison to FSIS
Jane Robens, Phone: 301-504-5381, Fax: 301-504-5476, E-mail: jfr@ars.usda.gov
National Program Leader, Food Safety and Health
USDA, ARS, NPS
BARC-West, Beltsville, MD 20705

Albany, CA	**Western Regional Research Center** 800 Buchanan Street Albany, CA 94710	James Seiber, CD 510-559-5600
Pathogen attachment, poultry, fruits and vegetables	**Food Safety and Health Research** Robert E. Mandrell, RL Stacy Pascoe, Secretary E-mail: mandrell@pw.usda.gov	 510-559-5829 510-559-5610 510-559-5948 Fax
Ronald G. Binder	rbinder@pw.usda.gov	510-559-5645
David L. Brandon	dbrandon@pw.usda.gov	510-559-5783
Amy Charkowski	amy@pw.usda.gov	510-559-5647
Mendel Friedman	mfried@pw.usda.gov	510-559-5615
William Gaffield	wg@pw.usda.gov	510-559-5979
William F. Haddon	haddon@pw.usda.gov	510-559-5803
Saima Kint	skint@pw.usda.gov	510-559-5767
William Miller	bmiller@pw.usda.gov	510-559-5992
Larry H. Stanker	stanker@pw.usda.gov	510-559-5829
Marian Wachtel	wachtel@pw.usda.gov	510-559-5957

	Process Chemistry and Engineering Research	
Advanced technologies, microorganisms and particulate removal	George Robertson , RL Esperanza Gonzalez, Secretary E-mail: egonzalez@pw.usda.gov	510-559-5866 510-559-5621 510-559-5818 Fax

Albany, (cont.)

Bradley Hernlem	hernlemb@pw.usda.gov	510-559-5937
Charles C. Huxsoll	chuxsoll@pw.usda.gov	510-559-5861
George Robertson	grobertson@pw.usda.gov	510-559-5866
Lee-Shin Tsai	lstsai@pw.usda.gov	510-559-5878

Ames, IA

National Animal Disease Center
2300 Dayton Avenue, P.O. Box 70
Ames, IA 50010

Keith Murray, CD
515-663-7201

Avian and Swine Respiratory Diseases Unit

Campylobacter, *Arcobacter* prevention	Richard B. Rimler, RL	515-663-7255
	Email: rrimler@nadc.ars.usda.gov	515-663-7458 Fax

Enteric Diseases & Food Safety Research

Salmonella, disease resistance, PCR, rapid detection, shiga-toxin *E. coli*, bovine model	Irene V. Wesley, Acting RL	515-663-7291
	E-mail: sbolin@nadc.ars.usda.gov	
	Sandy Johnson, Secretary	515-663-7244
	Email: sjohnson@nadc.ars.usda.gov	515-663-7458 Fax
StveR. Bolin	sbolin@nadc.ars.usda.gov	515-663-7244
Thomas A. Casey	tcasey@nadc.ars.usda.gov	515-663-7726
Steven A. Carlson	scarlson@nadc.ars.usda.gov	515-663-7612
Scott Hurd	shurd@nadc.ars.usda.gov	515-663-7672
Evelyn A. Nystrom	enystrom@nadc.ars.usda.gov	515-663-7376
Vijay K. Sharma	vsharma@nadc.ars.usda.gov	515-663-7279
Thomas J. Stabel	tstabel@nadc.ars.usda.gov	515-663-7292

Metabolic Diseases & Immunology Research Unit

Cryptosporidium parvum control	Ronald L. Horst, RL	515-663-7312
	Email: rhorst@nadc.ars.usda.gov	515-663-7669 Fax
James A. Harp	jharp@nadc.ars.usda.gov	515-663-7533
Mark A. Rasmussen	mrasmuss@nadc.ars.usda.gov	515-663-7350

Athens, GA

Richard B. Russell Agricultural Research Center
950 College Station Road, PO Box 5677, Athens, GA 30604

Poultry Microbiological Safety

Salmonella and *Campylobacter* control	Norman J. Stern, RL E-mail: nstern@ars.usda.gov	706-546-3516
	Donna Hinson, Secretary E-mail: hinsond@ars.usda.gov	706-546-3531
J. Stan Bailey	jsbailey@ars.usda.gov	706-546-3484
Nelson A. Cox	ncox@ars.usda.gov	706-546-3484
Stephen E. Craven	scraven@ars.usda.gov	706-546-3986
J. Eric Line	eline@ars.usda.gov	706-546-3522

Antimicrobial Resistance

Antimicrobial resistance	Paula J. Fedorka-Cray, RL pcray@ars.usda.gov	706-546-3305

Poultry Processing & Meat Quality Research

Poultry slaughter and processing, pathology control	Clyde E. (Gene) Lyon, RL E-mail: glyon@ars.usda.gov Rhonda Harper, Program Assistant E-mail: rharper@ars.usda.gov	706-546-3418 706-546-3633 Fax 706-546-3345
Judy W. Arnold	jarnold@ars.usda.gov	706-546-3515
Mark Berrang	mberrang@ars.usda.gov	706-546-3551
Richard J. Buhr	jbuhr@ars.usda.gov	706-546-3339
John A. Cason	jcason@ars.usda.gov	706-546-3360
J. Andra Dickens	adickens@ars.usda.gov	706-546-3205
Arthur Hinton, Jr.	ahinton@ars.usda.gov	706-546-3621
Kurt C. Lawrence	klawre@ars.usda.gov	706-546-3527
Richard J. Meinersman	rmeine@ars.usda.gov	706-546-3236
Sam D. Senter	ssenter@ars.usda.gov	706-546-3486
Lou L. Young	lyoung@ars.usda.gov	706-546-2416
W. R. (Bob) Windham	rwindh@ars.usda.gov	706-546-3513

Athens (cont.)	**Southeast Poultry Research Laboratory** P.O. Box 5657 934 College Station Road Athens, GA 30605	
	Food Safety Research	
S. enteritidis in chickens	David E. Swayne, LD, RL dswayne@seprl.usda.gov	706-546-3433 706-546-3161
Richard K. Gast	rkgast@arches.uga.edu	706-546-3445
Jean Guard-Petter	jgpetter@arches.uga.edu	706-546-3446
Peter S. Holt	pholt@asrr.arsusda.gov	706-546-3442
Bailey W. Mitchell	bmithcell@seprl.usda.gov	706-546-3443

Beltsville, MD	**Beltsville Agricultural Research Center** Beltsville Area 10300 Baltimore Avenue Beltsville, MD 20705-2350	Phyllis Johnson, AD 301-504-6078
	Soil Microbial Systems Laboratory	
Manure treatments, pathogen survival	Patricia D. Millner, RL Dorothy Talmud, Secretary pmillner@asrr.arsusda.gov	 301-504-8163
L. Sikora	lsikora@asrr.arsusda.gov	301-504-9384
J. Karns	jkarns@asrr.arsusdsa.gov	301-504-6493
W. Mulbry	wmulbry@asrr.arsusda.gov	301-504-6417
	Environmental Chemistry Laboratory	
Pathogen transport and dissemination from manure	Cathleen J. Hapeman, RL Jenny Hawkins, Secretary E-mail: jhawkins@asrr.arsusda.gov	301-504-6511 301-504-651
Daniel R. Shelton	dshelton@asrr.arsusda.gov	301-504-6582
Ali M. Sadeghi	asadeghi@asrr.arsusda.gov	301-504-6693
Allen R. Isensee	aisensee@asrr.arsusda.gov	301-504-5297
Greg W. McCarty	gmccarty@asrr.arsusda.gov	301-504-7401
James L. Starr	jstarr@asrr.arsusda.gov	301-504-6888

Beltsville (cont.)	**Biosystematics and National Parasite Collection Unit**	
	Eric P. Hoberg, RL	
	email: ehoberg@lpsi.barc.usda.gov	301-504-8588
		301-504-8979 Fax
Benjamin M. Rosenthal	brosenth@lpsi.barc.usda.gov	301-504-8530
	Horticultural Crops Quality Lab	
Microbiology	Ken Gross, RL	301-504-6128
fresh produce quality	email: kgross@asrr.arsusda.gov	
	Sophie Lau-Lopez, Secretary	301-504-5107 Fax
	email: lau-lops@ba.ars.usda.gov	301-504-6128
Arvind Bhagwat	abhagwat@asrr.arsusda.gov	301-504-5106
Craig Bloch	cbloch@asrr.arsusda.gov	301-504-6128
William S. Conway	wconway@asrr.arsusda.gov	301-504-6980
James McEvoy	jmcevoy@asrr.arsusda.gov	301-504-6983
Robert A. Saftner	rsaftner@asrr.arsusda.gov	301-504-5672
Bruce Whitaker	whitaker@asrr.arsusda.gov	301-504-6984
	Immunology & Disease Resistance Lab	
Toxoplasmosis	Joan K. Lunney, RL	301-504-8201
swine parasites	jlunney@lpsi.barc.usda.gov	301-504-5306 Fax
Joseph F. Urban, Jr.,	jurban@lpsi.barc.usda.gov	301-504-8765
Dante S. Zarlenga	dzarleng@lpsi.barc.usda.gov	301-504-8754
Ron Fayer	rfayer@lpsi.barc.usda.gov	301-504-8750
Mark Jenkins	mjenkins@lpsi.barc.usda.gov	301-504-8054
James Higgins	jhiggins@lpsi.barc.usda.gov	301-504-6443
	Instrumentation and Sensing Lab	
Development of techniques	Yud-Ren Chen, RL	301-504-8450
for inspection of poultry	Thelma Brack, Secretary	301-504-9466 Fax
carcasses	Email: ychen@asrr.arsusda.gov	
Kuanglin (Kevin) Chao	kchao@asrr.arsusda.gov	301-504-8450
Moon Soon Kim	mkim@asrr.arsusda.gov	301-504-8450
Yongliang Liu	lyonglia@asrr.arsusda.gov	301-504-8450
Patrick Mehl	pmehl@asrr.arsusda.gov	301-504-8450

Beltsville, (cont.)

	Meat Science Research Lab	
Hydrodynamic pressure treatment, cooked color in beef patties, antibiotics, dioxin metabolism	Morse B. Solomon, RL Ralphine Andrews, Secretary Email: ralphine@lpsi.barc.usda.gov	301-504-8400 301-504-8438 Fax
Bradford W. Berry	bberry@lpsi.barc.usda.gov	301-504-8994
Anisha M. Campbell	acampbell@lpsi.barc.usda.gov	301-504-8996
George F. Fries (collab.)	fries@lpsi.barc.usda.gov	301-504-9198
William A. Moats (collab.)	wmoats@lpsi.barc.usda.gov	301-504-8989
Morse B. Solomon	msolomon@lpsi.barc.usda.gov	301-504-8400
	Parasite Biology and Epidemiology Lab	
Trichinosis tests in swine and horses, toxoplasmosis epidemiology and control	Livestock and Poultry Sciences Institute Building 1040, Room 103, BARC-East H. Ray Gamble, RL Email: rgamble@lpsi.barc.usda.gov	301-504-8300 301-504-6273 Fax
J. P. Dubey	jdubey@lpsi.barc.usda.gov	301-504-8128 301-504-9222 Fax
Marcia L. Rhoads	mrhoads@lpsi.barc.usda.gov	301-504-8761
Dante S. Zarlenga	dzarleng@lpsi.barc.usda.gov	301-504-8754
	Center of Food Safety and Post-Harvest Technology	
Detection and treatment of *Listeria* and other bacteria in channel catfish	Mississippi State University Box 9805, Mississippi State, MS 39762 James Lindsay, NPS (ARS contact) e-mail: Ruth@Ra.MsState.edu	310-504-4674 601-325-3200 601-325-8728 Fax
Charles H. White	ruth@ra.msstate.edu	601-325-3200

Clay Center, NE	**U.S. Meat Animal Research Center**	
	P.O. Box 166 State Spur 18D	Dan B. Laster, CD
	Clay Center, NE 68933-0166	402-762-4109
	E-mail: laster@email.marc.usda.gov	402-762-4111 Fax

Clay Center (cont.)		
	Animal Health Research	
Salmonella control	William W. Laegreid, RL	402-762-4177
E. coli O157:H7 Mabs	Joan Rosch, Secretary	402-762-4180
	E-mail: rosch@email.marc.usda.gov	402-762-4375 Fax
Robert O. Elder	elder@email.marc.usda.gov	402-762-4392
James E. Keen	keen@email.marc.usda.gov	402-762-4343
William W. Laegreid	laegreid@email.marc.usda.gov	402-762-4177
Carol G. Chitko-McKown	mckown@email.marc.usda.gov	402-762-4372
	Meats Research Unit	
Beef and pork washing,	Mohammad Koohmaraie, RL	402-762-4221
microbial sampling	koohmaraie@email.marc.usda.gov	402-762-4149 Fax
	Marylin Bierman, Secretary	402-762-4222
	bierman@email.marc.usda.gov	
Elaine D. Berry	berry@email.marc.usda.gov	402-762-4225
G. A. Barkocy-Gallagher	gallagher@email.marc.usda.gov	402-762-4228
Mildred B. Rivera-Betancourt	rivera@email.marc.usda.gov	402-762-4226
Gregory R. Siragusa	siragusa@email.marc.usda.gov	402-762-4227
College Station, TX	**Food Animal Protection Research Lab**	
	2881 F&B Road	G. Wayne Ivie, LD
	College Station, TX 77845	409-260-9372
Analytical methodology,	**Food and Feed Safety Research Unit**	
immunochemistry,	Leon F. Kubena, Acting-RL	409-260-9484
poultry, swine	email: kubena@usda.tamu.edu	
Salmonella control	Faye W. Cook, Secretary	409-260-9484
	email: cook@ffsru.tamu.edu	409-260-9332 Fax
Robin C. Anderson	anderson@ffsru.tamu.edu	409-260-9317
Ross. C. Beier	beier@ffsru.tamu.edu	409-260-9441
James A. Byrd	byrd@ffsru.tamu.edu	409-260-9331
Donald E. Corrier	corrier@ffsru.tamu.edu	409-260-9484
Roger B. Harvey	harvey@ffsru.tamu.edu	409-260-9259
Carol K. Holtzapple	holtzapple@ffsru.tamu.edu	409-260-9263
Michael E. Hume	hume@ffsru.tamu.edu	409-260-9404

College Station (cont.)

David J. Nisbet	nisbet@ffsru.tamu.edu	409-260-9368
Michael H. Kogut, PL	kogut@ffsru.tamu.edu	409-260-9221
Richard L. Ziprin	ziprin@ffsru.tamu.edu	409-260-9302

Fargo, ND

Red River Valley Agricultural Research Center
1605 Albrecht Blvd.
PO Box 5674-Univ. Station
Fargo, ND 58105-5674

Biosciences Research Laboratory

Animal drug metabolism, dioxins in beef, milk and forage

Animal Metabolism-Agricultural Chemicals Research Unit

Gerald L. Larsen, RL		701-239-1231
Vicki J. Peterson, Secretary		701-239-1430 Fax
Email: larseng@fargo.ars.usda.gov		
Eric A. Garber	garbere@fargo.ars.usda.gov	701-239-1248
Heldur Hakk	hakkh@fargo.ars.usda.gov	701-239-1293
Janice K. Huwe	huwej@fargo.ars.usda.gov	701-239-1288
Nancy W. Shappell	shappeln@fargo.ars.usda.gov	701-239-1233
Weilin L. Shelver	shelverw@fargo.ars.usda.gov	701-239-1425
David J. Smith	smithd@fargo.ars.usda.gov	701-239-1238

Fayetteville, AR

Poultry Production and Product Safety Research Unit
G. Wayne Ivie, Acting RL (College Station, TX)

Rayma Cox, Secretary		501-575-2654
Email: pppsr@comp.uark.edu		501-575-4202 Fax
Janice M. Balog	jbalog@comp.uark.edu	501-575-6299
Geraldine R. Huff	grhuff@comp.uark.edu	501-575-7966
William E. Huff	huff@comp.uark.edu	501-575-2104

Logan, UT	**Poisonous Plant Research Lab**	
	1150 East 1400 North	
Natural toxins	Logan, UT 84341	
in poisonous plants	Lynn F. James, RL	435-752-2941
	Email: pprl@cc.usu.edu	
	Patsy Bradfield, Secretary	435-752-2941
	Email: pbradfld@cc.usu.edu	435-752-5681 Fax
Kip E. Panter	kpanter@cc.usu.edu	435-752-2941
Bryan L. Stegelmeier	bsteg@cc.usu.edu	435-752-2941
James A. Pfister	jpfister@cc.usu.edu	435-752-2941
Michael H. Ralphs	mralphs@cc.usu.edu	435-752-2941
Dale R. Gardner	gardnerd@cc.usu.edu	435-752-2941
Stephen T. Lee	stlee@cc.usu.edu	435-752-2941

Peoria, IL	**National Center for Agricultural Utilization Research**	
	1815 N. University Street	Peter Johnsen, CD
	Peoria, IL 61604	309-681-6541
	Food Quality and Safety Research	
Analytical methods,	Kathleen Warner, Acting RL	309-681-6555
supercritical fluids,	Doris J. Meinke, Office Program Manager	
fat and nutrient analysis	email: meinekedj@mail.ncaur.usda.gov	
J. King	kingjw@mail.ncaur.usda.gov	309-681-6203
J. Snyder	snyderjm@mail.ncaur.usda.gov	309-681-6236
F. Eller	ellerfj@mail.ncaur.usda.gov	309-681-6232
S. Taylor	taylorsl@mail.ncaur.usda.gov	309-681-6204
J. Teel	teelja@mail.ncaur.usda.gov	309-681-6218

Raleigh, NC	**Food Science Research Unit**	
	322 Schaub Hall, Box 7624, NCSU	
	Raleigh, NC 27695-7624	
	Henry P. Fleming, RL/LC	919-515-2979
	E-mail: hfleming@unity.ncsu.edu	
	Dora Dean Toler, Program Assistant	919-515-2979
	E-mail: doratole@unity.ncsu.edu	919-856-4361 Fax

Raleigh (cont.)

William M. Walter, Jr.	wmwalter@ncsu.edu	919-515-2979
Roger F. McFeeters	rfm@unity.ncsu.edu	919-515-2979
Fred Breidt	breidt@ncsu.edu	919-515-2979
Roger L. Thompson	rlt@unity.ncsu.edu	919-515-2979

Wyndmoor, PA

Eastern Regional Research Center

600 East Mermaid Lane
Wyndmoor, PA 19038-8551

John P. Cherry, CD
jcherry@ars.usda.gov
215-233-6595

Engineering Science Research Unit

Surface pasteurization	James C. Craig, RL Email: jcraig@arserrc.gov	215-233-6589
	Patricia A. Coyle, Secretary Email: pcoyle@ars.usda.gov	215-233-6589 215-233-6795 Fax
Michael F. Kozempel	mkozempel@arserrc.gov	215-233-6588
Neil Goldberg	ngoldberg@arserrc.gov	215-233-6590
Andrew J. McAloon	amcaloon@arserrc.gov	215-233-6619
David J. Geveke	dgeveke@arserrc.gov	215-233-6507

Food Safety Research Unit

Optimization attachment, advanced technologies, assurance microbiology	Donald W. Thayer, RL dthayer@arserrc.gov	215-233-6582
	Elizabeth Richardson Email: erichardson@ars.usda.gov	215-233-6582 215-233-6406 Fax
Walter Fiddler	wfiddler@arserrc.gov	215-233-6502
Vijay K. Juneja	vjuneja@arserrc.gov	215-233-6500
Steven J. Lehotay	lehotays@arserrc.gov	215-233-6433
Robert Maxwell	rmaxwell@arserrc.gov	215-233-6433
John Novack	novackj@arserrc.gov	215-233-3762
John W. Pensabene	jpensabene@arserrc.gov	215-233-6503
Kathleen Rajkowski	krajkowski@arserrc.gov	215-233-6440
Marilyn J. Schneider	schneiderm@arserrc.gov	215-233-6743
Christopher Sommers	sommersc@arserrc.gov	215-233-6582

Wyndmoor (cont.)

Microbial Biophysics and Biochemistry

Bacterial detection, biosensor	Shu-I Tu, RL	215-233-6611
	Helen Cassidy, Secretary	215-233-6611
	Email: stu@arserrc.gov	215-233-6581 Fax
Jeffery Brewster	jbrewster@arserrc.gov	215-233-6447
C. Gerald Crawford	cgcrawford@arserrc.gov	215-233-6628
Peter L. Irwin	pirwin@arserrc.gov	215-233-6420
Linda S. Y. Yu	lyu@arserrc.gov	215-233 3740

Microbial Biophysics and Biochemistry at Purdue Univ.
1140 Agricultural Administration Building
Agricultural Research Programs
Purdue University
West Lafayette, IN 47907-1140

Richard Linton, Food Safety Engineering Project Director
email: linton@purdue.edu 765-494-6481
Randy Woodson, Director of Agriculture Research Programs

Microbial Food Safety Research

E. coli 0157:H7	John B. Luchansky, RL	215-233-6620
Listeria, Clostridia,	Kathryn Clough, Secretary	215-233-6620
microbial growth,	Email: kclough@naa.ars.usda.gov	215-233-6581 Fax
mathematical modeling		or -233-6568
product safety		
Darrell Bayles	dbayles@arserrc.gov	215-233-6678
Saumya Bhaduri	sbhaduri@arserrc.gov	215-233-6521
Pina M. Fratamico	pfratamico@arserrc.gov	215-233-6525
Marjorie B. Medina	mmedina@arserrc.gov	215-233-6436
Samuel A. Palumbo	spalumbo@arserrc.gov	215-233-6740
Laura L. Zaika	lzaika@arserrc.gov	215-233-6655

Microbial Food Safety Research at UMES

Math modeling	Princess Anne, Md (Worksite)

of *Salmonella* on	John B. Luchansky, RL	215-233-6620
poultry products,	Patricia Shannon, Admin.Techn.	410-651-6526
DNA fingerprinting	email: pshannon@mail.umes.edu	410-651-6568 Fax
Thomas Oscar	toscar@mail.umes.edu	410-651-6062

Microbial Food Safety Research at DSU

	Delaware State University, De (Worksite)	
Virus detection in	John B. Luchansky, RL	215-233-6620
shell fish, marine	Patricia Shannon, Admin. Techn.	302-739-2016
vibrios	Pshannon@dsc.edu	302-739-5198 Fax
Gary Richards	grichard@dsc.edu	302-857-6419

Plant Science & Technology Research Unit

Decontaminating	Kevin B. Hicks, RL	215-233-6579
fresh produce	email: khicks@arserrc.gov	215-233-6406 Fax
	Jackie Kenny, Secretary	215-233-6580
	jkenny@naa.ars.usda.gov	
Bassam Annous	bannous@arserrc.gov	215-233-6797
Gerald M. Sapers	gsapers@arserrc.gov	215-233-6417
Ching-Hsing Liao	cliao@arserrc.gov	215-233-6471
William F. Fett	wfett@arserrc.gov	215-233-6418
Dike Ukuku	dukuku@arserrc.gov	215-233-6427

ALPHABETICAL LIST OF SCIENTISTS

Name	Internet E-mail	Phone
Anderson, Robin, C.	anderson@ffsru.tamu.edu	409-260-9317
Andrews, Ralphine	ralphine@lpsi.barc.usda.gov	301-504-8400
Annous, Bassam	bannous@arserrc.gov	215-233-6797
Arnold, Judy W.	jarnold@ars.usda.gov	706-546-3515
Bailey, J. Stan	jsbailey@ars.usda.gov	706-546-3484
Balog ,Janice M.	jbalog@comp.uark.edu	501-575-6299
Barkocy-Gallagher, G. A.	gallagher@email.marc.usda.gov	402-762-4228
Bayles, Darrell	dbayles@arserrc.gov	215-233-6678
Beier, Ross, C.	beier@ffsru.tamu.edu	409-260-9441
Berrang, Mark	mberrang@ars.usda.gov	706-546-3551
Berry, Elaine D.	berry@email.marc.usda.gov	402-762-4225
Berry, Bradford W.	bberry@lpsi.barc.usda.gov	301-504-8994
Bhaduri, Saumya	sbhaduri@arserrc.gov	215-233-6521
Bhagwat, Arvind	abhagwat@asrr.arsusda.gov	301-504-5106
Bierman Marylin	bierman@email.marc.usda.gov	402-762-4222
Binder, Ronald G.	rbinder@pw.usda.gov	510-559-5645
Bloch, Craig	cbloch@asrr.arsusda.gov	301-504-6128
Bolin, Steven R.	sbolin@nadc.ars.usda.gov	515-663-7244
Bradfield, Patsy	pbradfld@cc.usu.edu	435-752-2941
Brandon, David L.	dbrandon@pw.usda.gov	510-559-5783
Breidt, Fred	breidt@ncsu.edu	919-515-2979
Brewster, Jeffery	jbrewster@arserrc.gov	215-233-6447
Buhr, Richard J.	jbuhr@ars.usda.gov	706-546-3339
Byrd, James, A.	byrd@ffsru.tamu.edu	409-260-9331

Campbell, Anisha M.	acampbell@lpsi.barc.usda.gov	301-504-8996
Carlson, Steven A.	scarlson@nadc.ars.usda.gov	515-663-7612
Casey, Thomas A.	tcasey@nadc.ars.usda.gov	515-663-7726
Cason, John A.	jcason@ars.usda.gov	706-546-3360
Cassidy, Helen	hcassidy@arserrc.gov	215-233-6611
Chao, Kuanglin	kchao@asrr.arsusda.gov	301-504-8450
Charkowski, Amy	amy@pw.usda.gov	510-559-5647
Chen, Yud-Ren	ychen@asrr.arsusda.gov	301-504-8450
Cherry, John P.	jcherry@ars.usda.gov	215-233-6595
Chitko-McKown, C. G.	mckown@email.marc.usda.gov	402-762-4372
Clough, Kathryn	kclough@naa.ars.usda.gov	215-233-6620
Collins-Kelley, Lynda	lkelley@ars.usda.gov	706-546-3524
Conway, William S.	wconway@asrr.arsusda.gov	301-504-6980
Cook, Faye W.	cook@ffsru.tamu.edu	409-260-9484
Corrier, Donald. E.	corrier@ffsru.tamu.edu	409-260-9484
Cox, Rayma	pppsr@comp.uark.edu	501-575-2654
Cox, Nelson A.	ncox@ars.usda.gov	706-546-3484
Coyle, Patricia A.	pcoyle@ars.usda.gov	215-233-6589
Craig, James C.	jcraig@arserrc.gov	215-233-6589
Craven, Stephen E.	scraven@ars.usda.gov	706-546-3986
Crawford, C. Gerald	cgcrawford@arserrc.gov	215-233-6628
Crippen, T. C.	crippen@ffsru.tamu.edu	409-260-9374
Dickens, J. Andra	adickens@ars.usda.gov	706-546-3205
Dubey, J. P.	jdubey@lpsi.barc.usda.gov	301-504-8128
Elder, Robert O.	elder@email.marc.usda.gov	402-762-4392
Eller, F.	ellerfj@mail.ncaur.usda.gov	309-681-6232
Fayer, Ron	rfayer@lpsi.barc.usda.gov	301-504-8750

Fedorka-Cray, Paula J.	pcray@ars.usda.gov	706-546-3305
Fett, William F.	wfett@arserrc.gov	215-233-6418
Fiddler, Walter	wfiddler@arserrc.gov	215-233-6502
Fleming, Henry P.	hfleming@unity.ncsu.edu	919-515-2979
Fratamico, Pina M.	pfratamico@arserrc.gov	215-233-6525
Friedman, Mendel	mfried@pw.usda.gov	510-559-5615
Fries, George F.	fries@lpsi.barc.usda.gov	301-504-9198
Gaffield, William	wg@pw.usda.gov	510-559-5979
Gamble, H. Ray	rgamble@lpsi.barc.usda.gov	301-504-8300
Garber, Eric A.	garbere@fargo.ars.usda.gov	701-239-1248
Gardner, Dale R.	gardnerd@cc.usu.edu	435-752-2941
Gast, Richard K.	rkgast@arches.uga.edu	706-546-3445
Geveke, David J.	dgeveke@arserrc.gov	215-233-6507
Goldberg, Neil	ngoldberg@arserrc.gov	215-233-6590
Gonzalez, Esperanza	egonzalez@pw.usda.gov	510-559-5621
Gross, Ken	kgross@asrr.arsusda.gov	301-504-6128
Guard-Petter, Jean	jgpetter@arches.uga.edu	706-546-3446
Haddon, William F.	haddon@pw.usda.gov	510-559-5803
Hakk, Heldur	hakkh@fargo.ars.usda.gov	701-239-1293
Hapeman, Cathleen J.	chapeman@aasrr.arsusda.gov	301-504-6511
Harp, James A.	jharp@nadc.ars.usda.gov	515-663-7533
Harper, Rhonda	rharper@ars.usda.gov	706-546-3345
Harvey, Roger B.	harvey@ffsru.tamu.edu	409-260-9259
Hawkins, Jenny	jhawkins@asrr.arsusda.gov	301-504-6511
Hernlem, Bradley	hernlemb@pw.usda.gov	510-559-5937
Hicks, Kevin B.	khicks@arserrc.gov	215-233-6579
Higgins, James	jhiggins@lpsi.barc.usda.gov	301-504-6443

Hinson, Donna	hinsond@ars.usda.gov	706-546-3531
Hinton, Arthur Jr.	ahinton@ars.usda.gov	706-546-3621
Hoberg, Eric P.	ehoberg@lpsi.barc.usda.gov	301-504-8588
Holt, Peter S.	pholt@asrr.arsusda.gov	706-546-3442
Holtzapple, Carol K.	holtzapple@ffsru.tamu.edu	409-260-9263
Horst, Ronald L.	rhorst@nadc.ars.usda.gov	515-663-7312
Huff, William E.	huff@comp.uark.edu	501-575-2104
Huff, Geraldine R.	grhuff@comp.uark.edu	501-575-7966
Hume, Michael E.	hume@ffsru.tamu.edu	409-260-9404
Hurd, Scott	shurd@nadc.ars.usda.gov	515-663-7672
Huwe, Janice K.	huwej@fargo.ars.usda.gov	701-239-1288
Huxsoll, Charles C.	chuxsoll@pw.usda.gov	510-559-5861
Irwin, Peter L.	pirwin@arserrc.gov	215-233-6420
Isensee, Allen R.	aisensee@asrr.arsusda.gov	301-504-5297
Ivie, G. Wayne	gwivie@acs.tamu.edu	501-575-2654
James, Lynn F.	pprl@cc.usu.edu	435-752-2941
Jenkins, Mark	mjenkins@lpsi.barc.usda.gov	301-504-8054
Johnsen, Peter	pjohnsen@mail.ncaur.usda.gov	309-681-6541
Johnson, Sandy	sjohnson@nadc.ars.usda.gov	515-663-7244
Juneja, Vijay K.	vjuneja@arserrc.gov	215-233-6500
Karns, J.	jkarns@asrr.arsusdsa.gov	301-504-6493
Keen, James E.	keen@email.marc.usda.gov	402-762-4343
Kenny, Jackie	jkenny@naa.ars.usda.gov	215-233-6580
Kim, Moon Soon	mkim@asrr.arsusda.gov	301-504-8450
King, J.	kingjw@mail.ncaur.usda.gov	309-681-6203
Kint, Saima	skint@pw.usda.gov	510-559-5767
Kogut, Michael H.	kogut@ffsru.tamu.edu	409-260-9221

Koohmaraie, M.	koohmaraie@email.marc.usda.gov	402-762-4221
Kozempel, Michael F.	mkozempel@arserrc.gov	215-233-6588
Kubena, Leon F.	kubena@ffsru.tamu.edu	409-260-9249
Laegreid, William W.	laegreid@email.marc.usda.gov	402-762-4177
Larsen, Gerald L.	larseng@fargo.ars.usda.gov	701-239-1231
Laster, Dan B.	laster@email.marc.usda.gov	402-762-4109
Lau-Lopez, Sophie	lau-lops@ba.ars.usda.gov	301-504-6128
Lawrence, Kurt C.	klawre@ars.usda.gov	706-546-3527
Lee, Stephen T.	stlee@cc.usu.edu	435-752-2941
Lehotay, Steven J.	lehotays@arserrc.gov	215-233-6433
Liao, Ching-Hsing	cliao@arserrc.gov	215-233-6471
Lindsay, James	jal@ars.usda.gov	301-504-4674
Line, J. Eric	eline@ars.usda.gov	706-546-3522
Linton, Richard	linton@purdue.edu	765-494-6481
Liu, Yongliang	lyonglia@asrr.arsusda.gov	301-504-8450
Luchansky, John B.	jluchansky@arserrc.gov	215-233-6620
Lunney, Joan K.	jlunney@lpsi.barc.usda.gov	301-504-8201
Lyon, Clyde E. (Gene)	glyon@ars.usda.gov	706-546-3418
Mandrell, Robert E.	mandrell@pw.usda.gov	510-559-5829
Maxwell, Robert	rmaxwell@arserrc.gov	215-233-6433
McAloon, Andrew J.	amcaloon@arserrc.gov	215-233-6619
McCarty, Greg W.	gmccarty@asrr.arsusda.gov	301-504-7401
McEvoy, James	jmcevoy@asrr.arsusda.gov	301-504-6983
McFeeters, Roger F.	rfm@unity.ncsu.edu	919-515-2979
Medina, Marjorie B.	mmedina@arserrc.gov	215-233-6436
Mehl, Patrick	pmehl@asrr.arsusda.gov	301-504-8450
Meinersmann, Richard	rmeiners@ars.usda.gov	706-546-3236

Meinke, Doris J.	meinekedj@mail.ncaur.usda.gov	
Miller, William	bmiller@pw.usda.gov	510-559-5992
Millner, Patricia D	pmillner@asrr.arsusda.gov	301-504-8163
Moats, William A.	wmoats@lpsi.barc.usda.gov	301-504-8989
Mulbry, W.	wmulbry@asrr.arsusda.gov	301-504-6417
Murray, Keith	kmurray@nadc.ars.usda.gov	515-663-7201
Nisbet, David J.	nisbet@ffsru.tamu.edu	409-260-9368
Novack, John	novackj@arserrc.gov	215-233-3762
Nystrom, Evelyn A.	enystrom@nadc.ars.usda.gov	515-663-7376
Oscar, Thomas	toscar@mail.umes.edu	410-651-6062
Owens, Jane	posaa.saa.jowens@ars.usda.gov	706-546-3497
Palumbo, Samuel A.	spalumbo@arserrc.gov	215-233-6740
Panter, Kip E.	kpanter@cc.usu.edu	435-752-2941
Pascoe, Stacy	spascoe@pw.usda.gov	510-559-5610
Pensabene, John W.	jpensabene@arserrc.gov	215-233-6503
Pfister, James A.	jpfister@cc.usu.edu	435-752-2941
Pishko, E. Jane	pishko@ffsru.tamu.edu	409-260-9397
Rajkowski, Kathleen	krajkowski@arserrc.gov	215-233-6440
Ralphs, Michael H.	mralphs@cc.usu.edu	435-752-2941
Rasmussen, Mark A.	mrasmuss@nadc.ars.usda.gov	515-663-7350
Rhoads, Marcia L.	mrhoads@lpsi.barc.usda.gov	301-504-8761
Richards, Gary	grichard@dsc.edu	302-857-6419
Richardson, Elizabeth	erichardson@ars.usda.gov	215-233-6582
Rimler, Richard B.	rrimler@nadc.ars.usda.gov	515-663-7255
Rivera-Betancourt, M. B.	rivera@email.marc.usda.gov	402-762-4226
Robertson, George H.	grobertson@pw.usda.gov	510-559-5866
Rosch, Joan	rosch@email.marc.usda.gov	402-762-4180

Rosenthal, Benjamin M.	brosenth@lpsi.barc.usda.gov	301-504-8530
Sadeghi, Ali M.	asadeghi@asrr.arsusda.gov	301-504-6693
Saftner, Robert A.	rsaftner@asrr.arsusda.gov	301-504-5672
Sapers, Gerald M.	gsapers@arserrc.gov	215-233-6417
Schneider, Marilyn J.	schneiderm@arserrc.gov	215-233-6743
Seiber, James	jseiber@pw.usda.gov	510-559-5600
Senter, Sam D.	ssenter@ars.usda.gov	706-546-3486
Shannon, Patricia	pshannon@mail.umes.edu	410-651-6526
Shannon, Patricia	pshannon@dsc.edu	302-739-2016
Shappell, Nancy W.	shappeln@fargo.ars.usda.gov	701-239-1233
Sharma, Vijay K.	vsharma@nadc.ars.usda.gov	515-663-7279
Shelton, Dan R.	dshelton@asrr.arsusda.gov	301-504-6582
Shelver, Weilin L.	shelverw@fargo.ars.usda.gov	701-239-1425
Sikora, L.	lsikora@asrr.arsusda.gov	301-504-9384
Siragusa, Gregory R.	siragusa@email.marc.usda.gov	402-762-4227
Smith, David J.	smithd@fargo.ars.usda.gov	701-239-1238
Snyder, J.	snyderjm@mail.ncaur.usda.gov	309-681-6236
Solomon, Morse B.	msolomon@lpsi.barc.usda.gov	301-504-8400
Sommers, Christopher	sommersc@arserrc.gov	215-233-6582
Stabel, Thomas J.	tstabel@nadc.ars.usda.gov	515-663-7292
Stanker, Larry	ruth@ra.msstate.edu	601-325-3200
Stanker, Larry H.	stanker@usda.tamu.edu	409-260-9484
Starr, James L.	jstarr@asrr.arsusda.gov	301-504-6888
Stegelmeier, Bryan L.	bsteg@cc.usu.edu	435-752-2941
Stern, Norman J.	nstern@ars.usda.gov	706-546-3516
Swayne, David E.	dswayne@seprl.usda.gov	706-546-3433
Taylor, S.	taylorsl@mail.ncaur.usda.gov	309-681-6204

Teel, J.	teelja@mail.ncaur.usda.gov	309-681-6218
Thayer, Donald W.	dthayer@arserrc.gov	215-233-6582
Thompson, Roger L.	rlt@unity.ncsu.edu	919-515-2979
Toler, Dora Dean	doratole@unity.ncsu.edu	919-515-2979
Tsai, Lee-Shin	lstsai@pw.usda.gov	510-559-5878
Tu, Shu-I	stu@arserrc.gov	215-233-6611
Ukuku, Dike	dukuku@arserrc.gov	215-233-6427
Urban, Joseph F., Jr.	jurban@lpsi.barc.usda.gov	301-504-8765
Wachtel, Marian	wachtel@pw.usda.gov	510-559-5957
Walter, William M. Jr.	wmwalter@ncsu.edu	919-515-2979
Warner, Kathleen	kwarner@mail.ncaur.usda.gov	309-681-6555
Wesley, Irene V.	iwesley@nadc.ars.usda.gov	515-663-7291
Whitaker, Bruce	whitaker@asrr.arsusda.gov	301-504-6984
White, Charles H.	ruth@ra.msstate.edu	601-325-3200
Windham, William R.	bobw@athens.net	706-546-3513
Windham, W. R. (Bob)	rwindh@ars.usda.gov	706-546-3513
Young, Lou L.	lyoung@ars.usda.gov	706-546-2416
Yu, Linda S. Y.	lyu@arserrc.gov	215-233 3740
Zaika, Laura L.	lzaika@arserrc.gov	215-233-6655
Zarlenga, Dante S.	dzarleng@lpsi.barc.usda.gov	301-504-8754
Ziprin, Richard L.	ziprin@ffsru.tamu.edu	409-260-9302

INDEX